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VOL. 14, SEC. C. MAY, 1936 NUMBER 5

THE REACTION OF WHEAT HYBRIDS TO A SPRING FROST[1]

BY J. B. HARRINGTON[2]

Abstract

A moderate June frost proved highly efficient in the separation of wheat hybrid lines for resistance to spring frost. Eighty of 332 F_6 lines of H-44-24 × Reliance and fully 56 of 227 F_5 lines of (Reliance × Reward) × Reliance appeared to have the superior frost resistance of Reliance. Significant genetic differences in frost reaction were found among the lines of each of two new varieties.

Introduction

Cereal crop improvement has been going on for thousands of years and methods of breeding have advanced from primitive ways to the highly scientific technique of the present day. Until comparatively recent times the bringing together of widely different gene complexes through the hybridization of varieties and species found in widely separated regions of the earth was practiced infrequently. Now, however, such crosses form a distinct part of the modern breeding technique. This is particularly true in the case of the small grains. The old introduction method of improvement emphasized primarily the adaptation of a variety to a certain environment, and care was taken to obtain varieties from closely similar habitats. The new breeding technique, however, places major emphasis on character combinations and, in order to obtain a desired character, a variety quite lacking in general adaptation to the area under consideration may be used as a parent. Thus many of the hybrids possess various unsuitable attributes along with the desired character. The presence of these unfavorable attributes is not always evident or even suspected. Above all, these characters are not easy to avoid and sometimes, on account of linkage, are extremely difficult to eliminate.

An important example of an unfavorable character which accompanied a desirable one is susceptibility to damage from spring frosts. Resistance of spring wheat to spring frost has not been considered an important character, partly because its influence on the final outcome of a crop is obscure and partly because the well acclimated and introduced varieties used in the regions subject to late spring frosts are usually fairly resistant to cold.

Attention was drawn to the possible importance of spring frost resistance in cereals by Waldron (3) when he revealed the low frost resistance of Hope wheat as compared with the high resistance of some other varieties, and when he (Waldron 4) showed further that severe injury from spring frost had a

[1] *Manuscript received April 3, 1936.*
Contribution from the University of Saskatchewan, Saskatoon, Saskatchewan.
[2] *Professor of Field Husbandry.*

depressing effect on yield. Hope was produced by McFadden (2) of South Dakota by crossing Marquis with Yaroslav, an emmer wheat, for the purpose of combining the high resistance of emmer to black stem rust with the good qualities of Marquis. But frost susceptibility and some other unfavorable characters accompanied the rust resistance. In recent years Hope and a sister variety called H-44-24 have been used extensively in breeding programs in both Canada and the United States.

The importance of satisfactory tests of hybrid lines for resistance to frost is apparent. Comparative frost tests may be made in field nurseries or in controlled refrigeration chambers. Field tests are highly desirable but are difficult to bring about as it is largely a matter of chance whether a satisfactory low temperature condition occurs when the seedlings are in the very susceptible two- or three-leaf stage.

An exceptionally good opportunity to obtain critical data on the reaction of wheat hybrid lines to spring frost occurred on June 4, 1935, at Saskatoon, Saskatchewan. A fairly uniformly distributed frost of one to four degrees F. lasted for a period of four hours during the night. At this time a number of comparative plot tests of cereal varieties as well as several thousand rows of hybrid lines were in the two- and three-leaf stage. Notes were taken on the second and third days after the frost on many hundreds of plots and rows and the results proved extremely interesting.

The data from the various comparative plot tests of varieties were presented in a recent paper by the writer (1). The varieties ranged from highly frost resistant to highly frost susceptible ones in each crop. The comparative results on varieties of wheat showed a clear-cut relationship between ancestry and frost resistance.

In the present paper the summarized data from the hybrid lines will be presented. The data were obtained from two nurseries which were sown earlier than usual with the hope of obtaining a field test of comparative resistance to spring frost. The nurseries comprised several hundred F_5 and F_6 progeny plots of crosses involving both frost resistant and frost susceptible varieties of spring wheat. Check plots of the parental varieties were sown systematically throughout both nurseries.

Methods

The frost injury notes were taken on the hybrids three days after the frost occurred. A scale of 0 to 10 was used as follows: 0, all above ground parts of the seedling killed; 1, two leaves and culm severely injured; 2, two leaves severely and culm moderately injured; 3, two leaves severely and culm slightly injured; 4, two leaves severely injured; 5, one leaf severely and one moderately injured; 6, two leaves moderately, or one leaf severely and one slightly injured; 7, one leaf severely injured; 8, one leaf moderately injured; 9, one leaf slighlty injured; 10, no apparent injury. While the observations were on an individual plant basis a general average for all of the plants in a plot, that is for one line, was taken in each case.

Results

The frost injury data on 332 F_6 lines of the cross H-44-24 × Reliance are given in Table I. The mean of the lines was 8.0 ± .05 which was intermediate between the Reliance mean of 9.0 ± .10 and the H-44-24 mean of 7.2 ± .20. Each of the three means differed from the others by highly significant odds. No correlation whatever was found between date of emergence and frost resistance index and nearly all of the plants were in the two-leaf stage and approximately of the same age at the time of the frost. The results indicate that there are significant genetic differences among the lines. It is reasonably safe to consider that the 79 lines occurring in Classes 9 and 10 possess the superior frost resistance of Reliance.

TABLE I

DISTRIBUTION OF F_6 LINES OF THE CROSS H-44-24 × RELIANCE FOR INJURY DUE TO SPRING FROST AT SASKATOON IN 1935

Material	No. of plots	Distribution for degree of frost injury							Mean S.E.
		4	5	6	7	8	9	10	
F_6 lines	332		1	10	68	174	69	10	8.0 ± .05
Reliance	27					3	20	4	9.0 ± .10
H-44-24	27	1	1	3	10	11	1		7.2 ± .20

General mean = 8.01. S.E.$_s$ = 0.91.

TABLE II

DISTRIBUTION OF F_5 LINES OF THE CROSS (RELIANCE × REWARD) × RELIANCE FOR INJURY DUE TO SPRING FROST AT SASKATOON IN 1935

Material	No. of plots	Distribution for degree of frost injury							
		4	5	6	7	8	9	10	Mean S.E.
F_5 lines	227	5	14	30	45	77	51	5	7.5 ± .09
Reliance	21			1	4	9	6	1	8.1 ± .20
Reward	79	2	7	24	37	9			6.6 ± .10

General mean = 7.32. S.E.$_s$ = 1.35.

Table II shows frost injury data on 227 F_5 lines of the back-cross of Reliance × Reward on Reliance. The nursery where this material was grown was nearly 1000 feet from the one which furnished the data in Table I and the frost injury was more severe. The mean of the hybrid lines was 7.5 ± .09 while that of Reliance was 8.1 ± .20 and of Reward was 6.6 ± .10. Tests of significance show the three means to differ one from another by high odds. As with the Table I data, no correlation was found between date of emergence and degree of frost injury. The results indicate that genetic differences for frost reaction exist among the lines. Fully 56 of the lines appear to have the Reliance type of reaction and at least 49 show the Reward susceptibility to frost.

The two foregoing tables have demonstrated the separation of frost resistant and frost susceptible hybrid lines by means of a critical field test. Lacking a satisfactory frost resistance test, hybrid lines would be chosen without regard to frost resistance. In this way a new variety might lack uniformity as to frost resistance but this would not become apparent until a critical frost injury test was made. In 1935, at Saskatoon, 87 purified lines of three new stem-rust-resistant varieties of wheat were grown with some varieties used as checks in a randomized four replicate rod row plot test. The data obtained on resistance to the June 4 frost afforded an opportunity to note indications of genetic differences in frost resistance among the lines of two of the three varieties. The data are summarized in Table III.

TABLE III

SPRING FROST REACTION OF INDIVIDUAL PLANT LINES OF THREE NEW RUST RESISTANT VARIETIES OF SPRING WHEAT GROWN IN A RANDOMIZED FOUR REPLICATE ROD ROW PLOT TEST IN 1935

Material	No. of lines	Line distribution for frost injury*															
		4.2	4.5	4.8	5.1	5.4	5.7	6.0	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.4	Mean S.E.
Apex lines	25			2	1	2	2	2	9	3	3	1					6.2 ± .18
A 277 lines	14			3	4	2	2	2		1							5.4 ± .21
A 41 lines	48	4	7	24	6	3	2	1	1								4.9 ± .10
Apex										1							6.6 ± .49
A 277									1								6.3 ± .46
A 41						1											5.4 ± .40
Reliance																1	8.4 ± .62
Thatcher									1								6.3 ± .46
Marquis									1								6.3 ± .46
Reward				1													4.8 ± .35

* *Average of four replicates in each case.*
The standard error of a variety mean equals 7.4%.
χ^2 *tests:*
Apex lines vs. A 277 lines. $\chi^2 = 7.93, P = .02.$
A 277 lines vs. A 41 lines. $\chi^2 = 6.55, P = .01.$

The results reveal differences, which appear to be genetical, within the groups of lines. In Apex all of the lines rating 6.3 were less injured (odds of more than 21 to 1) than those rating 4.8. Sixteen of the 25 lines rated 6.3 or more. Lines rating 6.9 were less injured (odds of more than 45 to 1) than those in the 4.8 class, yet 4 lines rated from 6.9 to 7.2. These results show highly significant differences between lines with respect to frost reaction. In the A41 lines the distribution also deviates from the expected normal, though much less strikingly. In the A277 lines no significant deviation from normal expectation is shown.

It is worth noting also in Table III that there were significant differences between the line distributions of the three new varieties. All of these varieties originated from the cross (H-44-24 × Double Cross) × Marquis. Considering the parentage of H-44-24 and Double Cross, the three new hybrid varieties have inheritance ranging from the high frost resistance of Kanred, as represented by the Reliance reaction, to the relatively low resistance of H-44-24,

which is represented fairly well by the reaction of Reward (See 1). It is therefore to be expected that Apex, A277 and A41 differ from one another in frost reaction. These differences are only shown significantly in the reactions of the lines. Nevertheless the frost resistance order in which the three varieties occurred in their use as checks is the same as the order shown by the mean indices of the groups of lines.

Discussion

Owing to the present strong competition in the marketing of cereals, particularly wheat, it is necessary to have more efficient varieties as one means of reducing production costs. One mode of increasing the efficiency lies in adding disease resistance which is obtained usually from varieties introduced from distant parts of the world. Along with the disease resistance the introduced varieties bring undesirable characters. Frost susceptibility is one undesirable character contributed by the highly stem-rust-resistant emmer wheats.

As long as the wheats used on the western plains of North America were from hardy Russian stocks there was little need for paying attention to spring frost resistance as a varietal character. This situation changed when the hybridization method of breeding brought together the characters of distinctly unrelated wheats. The origination and use of Marquis wheat presumably introduced a measure of the frost susceptibility of the Indian parent, Hard Red Calcutta. Of late the origination of varieties resistant to stem rust by crossing common wheat with emmers has produced a further dose of spring frost susceptibility. Thus, while the increased disease resistance of the new varieties constitutes important progress, other characters possessed by these varieties may be definitely detrimental. It would now appear to be advisable to take steps to raise the general level of frost resistance. The high degree of frost resistance shown by Reliance and other varieties (1) derived from crosses with Kanred winter wheat indicates that a more extensive use of winter wheats in spring wheat breeding programs might be a very satisfactory procedure. In any event, it is obvious that adequate comparative tests for spring frost resistance are needed at the breeding institutions of the northern plains area of North America. It would seem that a plant breeding establishment should either be equipped with adequate refrigeration test chambers or else be able to have its new hybrid lines tested at some station which has such equipment.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

I. CROSSING RESULTS AND DESCRIPTION OF THE FIRST GENERATION HYBRIDS[1]

BY J. M. ARMSTRONG[2]

Abstract

Two species of tetraploid ($2n = 28$) and three varieties of hexaploid ($2n = 42$) wheat were crossed with *A. glaucum* ($2n = 42$), and *A. elongatum* ($2n = 70$), with an average crossing success of 18%. The seed obtained from tetraploid wheat × *A. glaucum* was slightly plumper and germinated better than that obtained from tetraploid wheat × *A. elongatum*. On the other hand, hexaploid wheat × *A. elongatum* gave decidedly plumper and better germinating seed than hexaploid × *A. glaucum*.

Grown under greenhouse conditions the F_1 hybrids proved to be self-sterile and perennial in habit, with hybrid vigor strongly marked. The hybrids were, in general, intermediate in morphological characters, but with somewhat more resemblance to *Agropyron* than to wheat. This dominance, whole or partial, was more noticeable in the *A. elongatum* than in the *A. glaucum* crosses. Dominance phenomena are discussed in relation to current theories.

Introduction

The practical possibilities of intergeneric crosses of the Hordeae have been emphasized by Dr. Meister, Director of the Central Station of Plant Breeding and Genetics at Saratov. The first successful crosses between *Triticum* and *Agropyron* were made in 1930 by N. V. Tzitzin. Since then the work in the U.S.S.R. has expanded rapidly and is now being carried on at several stations. The progress made has been reported by Tzitzin (5), Verushkine and Schekurdine (7) and Vakar (6). Their chief aim has been the creation of new forms of perennial wheat. For Canadian conditions the possibilities of obtaining new forms of forage crops by this method appear more attractive. Such new forms should be of considerable value in solving the soil-drifting problem of the prairie provinces. The present need is for a large-seeded forage crop which would be easy to establish and which would possess good soil-binding properties. Such a crop, besides restoring fibre to the soil, should furnish a good quality of feed grain in addition to fodder and pasture.

At the Central Experimental Farm during the summer of 1935, crosses were made between species of *Triticum* and *Agropyron*, and small populations of the various crosses were grown in the greenhouse during the past winter.

Description of Parents

The species of wheat and grass which were successfully crossed are shown in Table I. The wheat varieties were kindly supplied by the Cereal Division, Central Experimental Farm. They are pure line selections, highly homozygous for most morphological characters.

[1] *Manuscript received May 11, 1936.*
Contribution from the Division of Forage Plants, Central Experimental Farm, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron undertaken by the Dominion Experimental Farm and the National Research Council, Canada. Presented at the Ottawa meeting of the Royal Society of Canada, May, 1936.
[2] *Research assistant, Division of Forage Plants.*

TABLE I

SPECIES OF WHEAT AND *Agropyron* USED IN SUCCESSFUL CROSSES

Species	Variety	Polyploidy	Growth habit
Triticum dicoccum	Vernal Emmer	Tetraploid	Spring form
T. durum	Mindum	Tetraploid	Spring form
T. vulgare	Lutescens	Hexaploid	Spring form
T. vulgare	C.A.N. 1835	Hexaploid	Spring form
T. vulgare	Kharkov	Hexaploid	Winter form
Agropyron glaucum		Hexaploid	Perennial
A. elongatum		Decaploid	Perennial

C.A.N. 1835 requires a special explanation. It is a sixth generation segregate of the cross Pentad × Marquis, developed at the Winnipeg Rust Laboratory. Its grain is of high quality and it is quite rust-resistant.

While crosses were attempted between wheat and many species of *Agropyron*, only two species, *A. glaucum* and *A. elongatum*, were successful as male parents. These two species are indigenous to continental Europe and to Asia, but their forage possibilities have been tested at Ottawa for several years. They are tall-growing perennials of the bunch grass type with extensive, fibrous, root systems. Cross-pollination is usual although not obligatory. According to botanical descriptions these species are polymorphic, having well defined varieties and forms. Our limited observations have shown the existence of considerable variability. Both species have morphological features which render them highly drought-resistant. They are also claimed to be resistant to certain fungus diseases to which many species of wheat are susceptible. They have certain obvious faults however, such as coarse foliage and seed shattering.

A prospectus of the compatible species in the two genera indicates that, barring close genetic linkage, it should be possible to produce new varieties combining the desirable characters, perennial growth habit, drought and disease resistance, and vigorous root development of the grass parents with the characters, palatable foliage and large grain size of the wheat parents.

Crossing Results

The wheat species were used mainly as the female parents in the crosses, not only because the wheat varieties proved easier to emasculate and pollinate, but also because the *Agropyron* species furnished a more abundant supply of pollen. A few reciprocal crosses were attempted but with negative results.

It was observed that during the flowering period both *Agropyron* species shed their pollen on warm, bright afternoons. Large glassine bags were placed over a cluster of heads in the morning and in the afternoon a considerable quantity of pollen would be available for pollination. The pollen was placed in a petri dish or small envelope and applied to the stigmas of the emasculated wheat heads with a small camel's hair brush. For any given *Agropyron* species the flowering period lasted from seven to ten days.

To determine the error to be expected in crossing, a number of heads of each wheat variety were emasculated without subsequent pollination. Table II gives the results of this test. Out of a total of 2,645 flowers emasculated but not pollinated 19 flowers set seed, giving an error of 0.7%.

TABLE II

CROSSING ERROR AS DETERMINED BY EMASCULATION WITHOUT POLLINATION

Female parent	No. of flowers emasculated	No. of seeds set	Per cent error
Mindum	716	10	1.4
Vernal	196	2	1.0
C.A.N. 1835	637	0	0.0
Lutescens	942	7	0.7
Kharkov	154	0	0.0
Total	2645	19	0.7

The species of *Agropyron* which did not cross successfully with wheat are given in Table III. Nine such species were used in 34 combinations with an average of 193 flowers per combination. From the total of 9,597 flowers used in these crossing attempts, 36 seeds were obtained.

TABLE III

SPECIES OF *Agropyron* WHICH DID NOT CROSS SUCCESSFULLY WITH *Triticum*

Male parents	Female parents									
	Mindum		Vernal		Kharkov		Lutescens		C.A.N. 1835	
	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained
A. desertorum	118	0	76	0	71	0	360	1	320	0
A. dasystachyum	239	0	—	—	—	—	120	1	575	1
A. caninum	78	1	—	—	—	—	142	1	183	1
A. imbricatum	40	0	64	4	—	—	40	0	60	1
A. repens	300	1	558	8	108	0	256	2	180	1
A. cristatum (Fairway)	280	0	286	6	156	0	460	2	420	0
A. cristatum (Commercial)	200	0	160	0	155	1	178	0	220	2
A. obtusiusculum	56	0	40	2	—	—	—	—	58	0
A. Richardsonii	40	0	—	—	—	—	—	—	—	—
Total	1351	2	1184	20	490	1	1556	7	2016	6
Per cent seeds obtained	—	0.15	—	1.69	—	0.20	—	0.45	—	0.30

Assuming these seeds to be of hybrid origin, the crossing success would be 0.54%, which is lower than the crossing error given in Table II. The possibility of selfing was further indicated by the plumpness of the seeds in question. In order to remove any doubt the seed was sown and the plants were grown

to maturity in the greenhouse. All proved to be wheat plants. The failure to obtain crosses between these species of *Agropyron* and wheat is clearly due to incompatibility. The negative results obtained agree with those of the U.S.S.R. investigators.

Table IV gives the results of the successful crosses of wheat with *A. glaucum* and *A. elongatum*. Two strains of *A. elongatum* were used and the crossing results have been kept separate in the table. Considering the results as a whole, a total of 9,648 flowers were pollinated and 1,784 seeds were obtained giving a crossing success of 18.5%. Deducting the crossing error of Table II, the true success would be slightly under 18%.

TABLE IV

SUCCESSFUL CROSSES

Female parents	Male parents								
	A. glaucum			*A. elongatum* No. 820			*A. elongatum* No. 1083		
	Flowers pollinated	Seeds obtained	Per cent success	Flowers pollinated	Seeds obtained	Per cent success	Flowers pollinated	Seeds obtained	Per cent success
Vernal	1196	414	34.6	1391	538	38.7	196	3	1.5
Mindum	1224	394	32.2	345	11	3.2	164	12	7.3
Kharkov	1041	122	11.7	239	25	10.5	328	45	13.7
Lutescens	1012	85	8.4	460	10	2.2	228	37	16.2
C.A.N. 1835	1099	70	6.5	268	16	6.0	457	2	.4
Total	5572	1085	19.5	2703	600	22.2	1373	99	7.2

In the crosses with *A. glaucum* a definitely higher degree of success was obtained with the two tetraploid species in comparison with the three hexaploid varieties. Of the latter, Kharkov, the winter form, crossed the most successfully although the difference is not sufficient to indicate a greater compatibility.

In the crosses with *A. elongatum*, Vernal gave a very high and a very low crossing success with the two *A. elongatum* strains, No. 820 and No. 1083, respectively. Lutescens, on the contrary, crossed much more readily with Strain No. 1083 than with Strain No. 820. The differences in both cases are so marked that there is little doubt of their significance. In the case of the other wheat species, about the same degree of compatibility with the two *A. elongatum* strains is apparent. The results would seem to indicate that the two strains of *A. elongatum* differ in their compatibility with certain varieties of wheat. Tzitzin (5) also reported that the different polymorphic forms of *A. glaucum* varied widely in their crossibility with various wheats.

Relative Plumpness of Wheat and Hybrid Seed

Table V gives the weight per ten kernels for the various crosses in comparison to the wheat parents. Clear-cut results in regard to seed plumpness are apparent and can be briefly summarized. Tetraploid wheat gave slightly

plumper seed when crossed with *A. glaucum* than when crossed with *A. elongatum*. In the Vernal crosses the difference is not very strongly marked, but the large number of crossed seeds obtained enabled us to demonstrate a statistical difference. In the crosses with the hexaploid wheats the condition is reversed. The plumper seed was obtained from the *A. elongatum* crosses. While the number of crossed seed was not high enough to permit a statistical treatment yet the marked difference in kernel weight and the appearance of the seed (Plate I) show that this difference is highly significant.

TABLE V

MEAN WEIGHT OF HYBRID SEED IN COMPARISON TO SELFED SEED OF WHEAT PARENTS

Material	Mean weight of 10 kernels, gm.	No. of kernels weighed
Vernal	.410	500
Vernal × *A. glaucum*	.194 ± .0054	250
Vernal × *A. elongatum*	.159 ± .0068	250
Mindum	.482	500
Mindum × *A. elongatum*	.167	23
Mindum × *A. glaucum*	.240 ± .0560	250
Kharkov	.304	500
Kharkov × *A. glaucum*	.057	122
Kharkov × *A. elongatum*	.109	70
Lutescens	.339	500
Lutescens × *A. glaucum*	.071	85
Lutescens × *A. elongatum*	.167	47
C.A.N. 1835	.280	500
C.A.N. 1835 × *A. glaucum*	.064	70
C.A.N. 1835 × *A. elongatum*	.103	18

In tetraploid wheat × *A. glaucum*, the hybrid seed is approximately one-half the weight of the seed of the respective wheat parents while in tetraploid wheat × *A. elongatum* it is about one-third. In hexaploid wheat × *A. glaucum* the hybrid seed is about one-fifth the kernel weight of the wheat parents while the same wheats crossed with *A. elongatum* gave hybrid seed about one-third to one-half the weight of seed of the wheat parents.

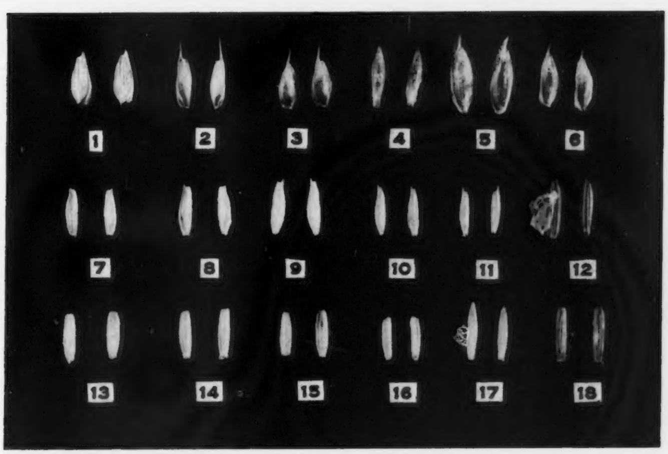
Germination of Hybrid and Parent Seed

Prior to the establishment of the F_1 populations in the greenhouse, germination tests were run on the parent and hybrid seed. The seeds were placed on sterilized quartz sand in petri dishes and the sand was kept moist with a 10% nutrient solution. The temperature was maintained at 65°–70° F. By this procedure it was hoped to promote development of the seedlings from the badly shrunken seeds as well as to make observations more convenient. Notes were taken daily on the rate of growth, rootlet number and percentage germination.

On the whole the germination was quite good, six of the crosses germinating more than 80%. By comparing Tables V and VI it will be seen that the plumper hybrid seed gave more satisfactory germination, this being most marked in the crosses involving *T. vulgare* varieties.

PLATE I

A



B

A. *Hybrid seed in comparison to seed of wheat parents.* 1. *Vernal* × *A. glaucum.* 2. *Vernal.* 3. *Vernal* × *A. elongatum.* 4. *Kharkov* × *A. glaucum.* 5. *Kharkov.* 6. *Kharkov* × *A. elongatum.* 7. *Lutescens* × *A. glaucum.* 8. *Lutescens.* 9. *Lutescens* × *A. elongatum.*

B. *Secondary glumes of wheat, Agropyron and hybrids from plants grown in the greenhouse with the exception of No. 5 which was field-grown.* 1. *Lutescens.* 2. *Kharkov.* 3. *C.A.N. 1835.* 4. *Vernal.* 5 and 6. *Mindum.* 7. *Lutescens* × *A. glaucum.* 8. *Kharkov* × *A. glaucum.* 9. *C.A.N. 1835* × *A. glaucum.* 10. *Vernal* × *A. glaucum.* 11. *Mindum* × *A. glaucum.* 12. *A. glaucum.* 13. *Lutescens* × *A. elongatum.* 14. *Kharkov* × *A. elongatum.* 15. *C.A.N. 1835* × *A. elongatum.* 16. *Vernal* × *A. elongatum.* 17. *Mindum* × *A. elongatum.* 18. *A. elongatum.*

TABLE VI

GERMINATION TESTS OF PARENTS AND F_1 HYBRID SEED

Material	No. of seeds sown	No. of seeds germinated	Germination, %	Times in days	Number of rootlets of germinating seeds						Greenhouse mortality
					5	4	3	2	1	0	
Vernal	75	75	100	4–5	72	3					0
Mindum	75	75	100	5–6	70	5					0
Kharkov	75	75	100	4–5	60	9	6				0
Lutescens	75	75	100	4–5	75						0
C.A.N. 1835	75	75	100	4–5	69	3	3				0
A. glaucum	96	86	90	8			55	23	8		0
A. elongatum No. 820	100	97	97	8			8	42	47		0
A. elongatum No. 1083	100	95	95	8			4	29	62		0
Vernal × *A. glaucum*	57	53	93	4–6		1*	52				0
Vernal × *A. elongatum*	59	51	87	4–6			41	9	1		0
Mindum × *A. glaucum*	57	55	96	4–5			53	1	1		1
Mindum × *A. elongatum*	19	9	47	5–7			9			1**	2
Kharkov × *A. glaucum*	63	40	64	6–9			5	16	19		1
Kharkov × *A. elongatum*	46	41	89	5–9			19	16	6		3
Lutescens × *A. glaucum*	50	31	62	5–8			3	4	24		5
Lutescens × *A. elongatum*	23	21	91	4–8			16	5			2
C.A.N. 1835 × *A. glaucum*	67	8	12	5–8					8		0
C.A.N. 1835 × *A. elongatum*	17	14	88	5–8			5	5	4		0

**This seedling had 2 plumules. **This seedling possessed a plumule.*

The cross C.A.N. 1835 × *A. glaucum* gave the very low germination of 12%. Upon dissecting the seeds that failed to germinate it was found that they were germless or completely lacking in embryos. This cross constitutes therefore an interesting case in the failure of double fertilization. Apparently at fertilization one male gamete fused with the polar nuclei to initiate endosperm development, but the other male gamete failed, in 88% of the cases, to effect fertilization of the egg.

A few other irregularities were observed in the germination tests. One seed from the cross Vernal × *A. glaucum* developed two plumules and four rootlets. This seedling subsequently developed into a normal F_1 plant. In another case a seed had a well developed plumule but no rootlets. A few seeds showed the reverse condition of one or more rootlets but no plumule. In other seeds the primordia ruptured the seed coat, but did not develop any further.

In number of seminal roots and in time required for germination, some significant differences were apparent. In wheat, all the seeds had three rootlets on the third day of the test, and by the fourth or fifth day two more rootlets had usually developed. A few seeds, particularly those of Kharkov, failed to develop the full complement of five rootlets. The grass species were slower to germinate than the wheat, and at the end of eight days had developed only one to three rootlets. The average rootlet number for *A. glaucum* was 2.5 and for the two strains of *A. elongatum* No. 820 and No. 1083 it was 1.6 and 1.4 respectively. In the time required for germination, the hybrid seed more closely approximated the wheat than the grass parents, although there

was less uniformity. In rootlet number they tended towards the grass parents since in no case were more than three rootlets per seed produced. It may be seen from Table VI that in the *T. vulgare* × *A. glaucum* crosses, which were characterized by very shrunken seeds, the majority of the seedlings had but one or two rootlets while in the *T. vulgare* × *A. elongatum* crosses, which gave plumper seed, most of the seedlings had two or three rootlets. If the degree of endosperm shrivelling had no effect on rootlet number the reverse condition would be expected, since *A. glaucum* has a higher rootlet number than *A. elongatum*. It may be concluded, therefore, that the factor or factors determining the rootlet number of the *Agropyron* parents is dominant over the allelomorphic factor in the wheat parents, but that the expression of this factor is modified by the endosperm condition of the hybrid seed.

Tzitzin reported that the hybrid seedlings which started with one rootlet invariably died before reaching maturity. Our results do not entirely agree with those of Tzitzin. The mortality noted in the last column of Table V was among the original one-rooted plants which died before there was any secondary root development, but, nevertheless the majority of one-rooted seedlings developed into normal plants.

Description of F_1 Plants

Growth and General Description

The F_1 seedlings, together with some parental material, were planted in a cool section of the greenhouse (50° F.) at the end of September. The hybrids tillered profusely, resembling the grass parents in this respect. Eight weeks after sowing, the hybrids and grass parents showed no indication of flowering stems, although all the wheats with the exception of Kharkov had done so. This winter habit was successfully broken by raising the temperature to 65°F. and exposing the plants to continuous light. Spike emergence of the hybrids commenced at the latter end of January and each plant flowered approximately two weeks after spike emergence.

Upon flowering, the lemma and palea separate widely and remain open ten days or longer. This type of behavior, characteristic of the open-pollinated *Agropyron* parents, may not be strictly inherited in the hybrids but may be due in part to sterility.

The female reproductive organs were normal in appearance, but the anthers, while appearing normal in size and color, failed to dehisce. Microscopic examination indicated about 5% good pollen. Complete self-fertility was found to be the rule in all the crosses.

One partially fertile hybrid occurred, however, in the C.A.N. 1835 × *A. elongatum* cross. In this plant the anthers dehisced normally and possessed 50–60% good pollen. Each head set about eight seeds which bore a general resemblance to those of the wheat parent.

All F_1 plants proved to be perennial like the *Agropyron* parents. The degrees of winter hardiness associated with this perennial growth habit will require tests this coming winter. Tzitzin found that the hybrids of *A. glaucum* with

spring wheat are much less frost-resistant than the corresponding hybrids with *A. elongatum* and that the hybrids with winter wheat were the hardiest of all. The dominance of the perennial habit in these crosses is of extreme importance not only because it permits the hybrids to be carried on indefinitely but also because, by means of cloning, a series of replicated experiments can be carried out to measure such characters as drought, frost and disease resistance.

Dominance Phenomena in the F_1 Hybrids

Eighteen pairs of characters were chosen for study. These were easily distinguishable in most of the parents. Some distinguish all the wheat varieties used from the *Agropyron* species, *e.g.*, habit (annual or perennial), spike compactness and number of rootlets in the seminal system; others, such as stem hollowness and width of glume, vary with one or both genera. The facts of dominance of the latter type of character were indeterminate in certain of the crosses.

Table VII shows the results of classifying the ten F_1 populations as to each of these 18 characters. The wheat condition is denoted by T, the *Agropyron* condition by A, intermediate by I, intermediate but more like wheat by IT and intermediate but more like *Agropyron* by IA.

Investigators in the field of *Triticum* × *Agropyron* hybridization have commented on the condition of dominance in the F_1 hybrids. Verushkine and Shechurdine (7) reported that "in all cases the F_1 plants showed the clear dominance of characters of the Couch grass and only a few plants occupied an intermediate position with regard to the character of their ear structure." Vakar (6) found that the dominance of the Couch grass characters was well defined in the hybrids. Tzitzin (5) on the other hand considered that in general the F_1 hybrids were intermediate with somewhat more resemblance to *Agropyron* than to wheat. An examination of the condition of dominance for the group of characters in Table VII confirms the latter author's observation. While it is true that there are more characters exemplifying dominance of the *Agropyron* than of the wheat parents, there is a large residue of intermediate characters.

The group of characters studied in which there is a clear dominance of the *Agropyron* parents will be dealt with first. Habit and the seminal root system have already been discussed and the dominance of the grass parents shown.

The time elapsing from spike emergence until anthesis differed markedly in the parents and hybrids. In wheat this period occupies four to six days and in the *Agropyron* 14–18 days. The hybrids closely resemble the *Agropyron* parents in this respect. Development during this period is not identical in wheat and *Agropyron*, as is clearly indicated by the different stages at which cytological preparations of the pollen mother cells must be taken. In both hybrids and the *Agropyron* parents three or four spikelets at the top of the spike must be showing clear of the sheath to be at the right stage for meiotic divisions, while the wheat species are at the right stage when the

TABLE VII

FREQUENCIES OF VARIOUS DEGREES OF DOMINANCE OF CHARACTERS IN TEN F_1 POPULATIONS OF *Triticum* × *Agropyron* CROSSES

F_1 population	Habit	Seminal root system	Spike density	Awning	Glume width	Beak	Keel	Barbs on keel	Glume adherence	Hollowness of culm	Leaf width	Number of leaf ridges	Prominence of ridges	Leaf Hairiness	Leaf scabrousness	Leaf posture	Waxiness	Time from spike emergence to anthesis	Frequencies of various degrees of dominance				
																			T	1 T	I	I A	A
Lutescens × *A. glaucum*	A	A	I	–	IA	I	IT	T	A	A	IT	A	IA	IT	I	–	IA	A	1	3	3	2	6
Lutescens × *A. elongatum*	A	A	IA	–	I	A	IA	I	A	A	I	I	IA	T	I	A	–	A	1	0	5	3	7
C.A.N. 1835 × *A. glaucum*	A	A	I	I	I	I	IT	IT	A	IA	I	IA	IA	T	I	–	A	A	1	2	6	3	5
C.A.N. 1835 × *A. elongatum*	A	A	IA	A	I	A	IA	I	A	A	A	IA	A	I	I	A	–	A	0	0	4	3	10
Kharkov × *A. glaucum*	A	A	I	I	I	I	IT	IT	A	I	IT	IA	I	I	I	–	A	A	0	3	8	1	5
Kharkov × *A. elongatum*	A	A	IA	A	I	A	IA	I	A	A	IA	IA	A	I	I	A	–	A	0	0	4	4	9
Mindum × *A. Glaucum*	A	A	I	I	I	I	IT	IT	A	I	IT	I	I	I	I	–	A	A	0	3	9	0	5
Mindum × *A. elongatum*	A	A	IA	IA	I	A	IA	IT	A	–	A	I	A	A	IA	A	–	A	0	1	2	4	9
Vernal × *A. glaucum*	A	A	I	I	–	I	IT	I	–	–	A	I	I	I	IA	–	–	A	0	1	7	1	4
Vernal × *A. elongatum*	A	A	IA	A	A	A	IA	I	–	–	A	IA	A	I	A	A	IA	A	0	0	2	4	10

spike shows only a barely perceptible swelling within the sheath. Consequently when the wheat spike emerges from the sheath the gametophytic phase is fairly far along, while the *Agropyron* species pass through the whole gametophytic stage after emergence from the sheath. The close agreement of the *Agropyron* parents and hybrids with respect to this part of the developmental cycle is fairly definitive of the whole cycle.

Glume adherence is another character for which the *Agropyron* parents showed complete dominance. The character depends mainly on the degree of development of the collar at the base of the glume. In the two *Agropyron* species and *T. dicoccum*, the outer glumes adhere closely to the flowering glumes while in the remaining wheat species the glumes adhere loosely. The expression of this character determines the threshing qualities of the species.

Glaucousness or waxy bloom, a character which imparts a dull, bluish-gray color to the plants previous to the ripening period, is characteristic of *A. glaucum* and is also present in *T. dicoccum.* In the four crosses in which this character is brought in by the *Agropyron* parent it is wholly or partially dominant, but in the cross in which it is brought in by the wheat parent, absence of waxiness is partially dominant.

The leaf blade attitude is very characteristic in *A. elongatum*, being of the "flag leaf" type. During the early growth stages the leaf blades are semi-vertical in attitude while on the culms they are retrorse. This character probably depends upon the interaction of several factors. In the five wheat × *A. elongatum* crosses the leaf attitude of the *Agropyron* parent is completely dominant.

Most of the characters for which intermediacy is exhibited in the F_1 hybrids are quantitative, *e.g.*, spike density, glume width, leaf width and leaf scabrousness. Partial dominance of the *Agropyron* parents is frequent, however, for even this type of character.

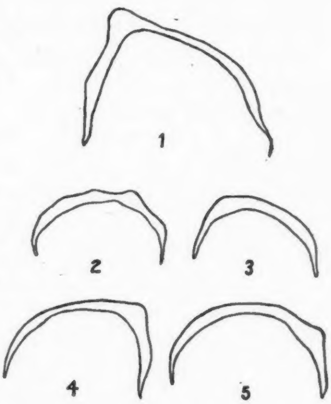
The most striking feature in this study is the contrasting degrees of dominance exhibited by the two sets of crosses, *Triticum* × *A. glaucum* and *Triticum* × *A. elongatum* for certain characters. This may be exemplified by the two characters, awning and the keel of the secondary glume.

All the wheat varieties except Lutescens are awned types, while the two *Agropyron* species are awnless. In the crosses of *A. glaucum* parentage all the hybrids are strongly tip-awned; that is, the intermediate condition prevails. In the crosses of *A. elongatum* parentage, on the other hand, the hybrids are completely awnless with the exception of *T. durum* × *A. elongatum* in which short tip awns are present on the lemmas of the upper spikelets.

Figs. 1–5 illustrate the keel condition in the parents and hybrids of the Karkov crosses. The cross sections were taken equidistant from the tip and base of the secondary glumes. In Kharkov the keel is strongly developed with the face and inner side of the glume approximately at right angles. In the *Agropyron* species the keel is weakly developed and the face and inner

side tend to be rounded without subtending a definite angle. The F_1 hybrids of Kharkov × *A. glaucum* tend to resemble the wheat parent both in the keel development and the angle of the face and side while in the F_1 of Kharkov × *A. elongatum* the keel is distinctly more like *A. elongatum.*

Other characters for which *A. elongatum* seems to exert a stronger influence than does *A. glaucum* are spike density, beak type and leaf width. The total effect is that the *A. elongatum* hybrids are distinctly more *Agropyron*-like than the *A. glaucum hybrids.*



FIGS. 1–5. *Cross-sections of secondary glumes drawn with the aid of a camera lucida.* 1. *Kharkov.* 2. *A. glaucum.* 3. *A. elongatum.* 4. *Kharkov × A. glaucum.* 5. *Kharkov × A. elongatum.*

Several theories devised to explain the phenomena of dominance have been advanced in recent years by various geneticists. Fisher (1) has advanced the theory of the evolution of dominance. His theory rests on the known frequent occurrence of mutations which are recessive to the wild type and deleterious to the organism. He believes that natural selection, acting over long periods of time, has operated to select appropriate modifying factors to make the heterozygotes equally viable to the homozygous wild type and indistinguishable from it.

Fisher's theory of the modification of a character to produce dominance implies that the heterozygotes were originally less viable than the wild type. This lack of viability is not found in the type of character dealt with in Table VII. Many species and varieties of wheat and *Agropyron* are known which possess the extreme alternative characters in awning, glume shape, beak length and spike density without any differential vigor being associated with them. Hence the dominance phenomena in the wheat × *Agropyron* hybrids cannot be adequately explained on Fisher's theory.

Wright (8) considers that it is unnecessary to formulate a theory for the evolution of dominance, but that final character expression depends upon the degree of completion of a chain of physiological processes under genic control. He postulates that the genes act through the control of specific catalysts, enzymes, and that the rate of any physiological process in the chain depends upon the proportion of catalyst to substrate. If the substrate is low in amount and the catalyst in excess, the further increase of the amount of the catalyst should exhibit extreme dominance. Conversely, if the substrate is in excess and the catalyst is low in amount the catalyst will be kept continually in combination and the gene will exhibit intermediacy.

This theory of the control of rate by limiting factors seems to be especially applicable in explaining the different degrees of dominance exhibited for

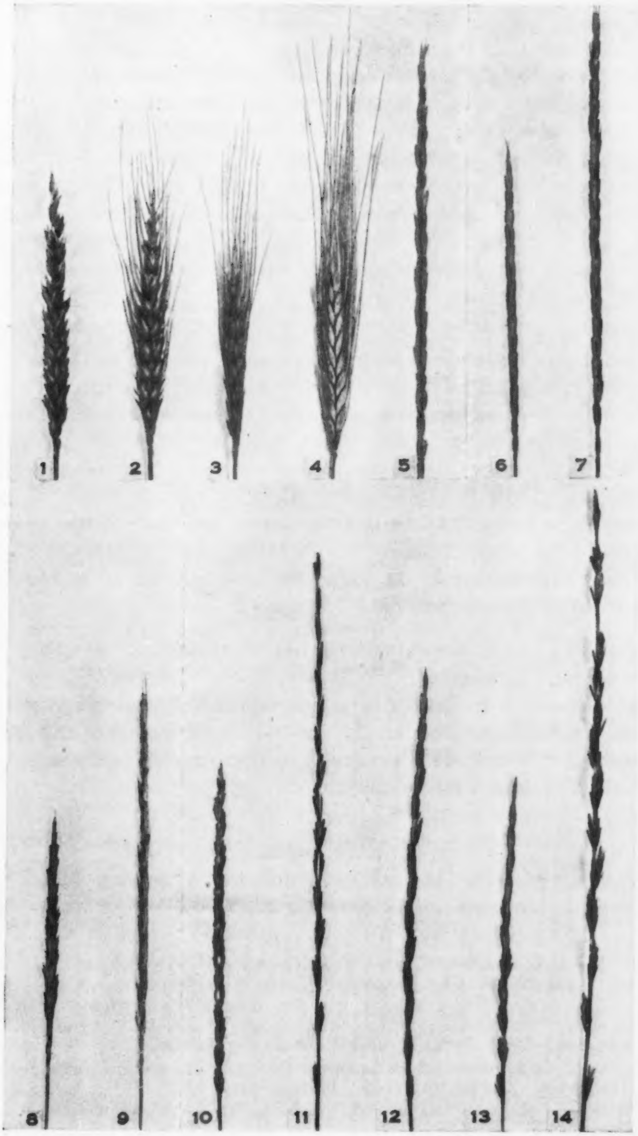


FIG. 6. *Heads of wheat and Agropyron parents and F_1 hybrids.* 1. *Lutescens.* 2. *Kharkov.* 3. *C.A.N. 1835.* 4. *Vernal.* 5. *A. glaucum.* 6. *Lutescens × A. glaucum.* 7. *Kharkov × A. glaucum.* 8. *C.A.N. 1835 × A. glaucum.* 9. *Vernal × A. glaucum.* 10. *Lutescens × A. elongatum.* 11. *Kharkov × A. elongatum.* 12. *C.A.N. 1835 × A. elongatum.* 13. *Vernal × A. elongatum.* 14. *A. elongatum.*

certain characters in the wheat × *A. glaucum* and wheat × *A. elongatum* crosses. *A. glaucum* is a hexaploid species ($2n = 42$) and *A. elongatum*, a decaploid ($2n = 70$), and on *a priori* grounds it might be presumed that there is a greater replication of genes affecting the same character in the latter species than in the former. Peto (3) has presented cytological evidence to show that *A. glaucum* is characterized by fairly regular bivalent formation at the first reduction division in the pollen mother cells while *A. elongatum* is characterized by frequent quadrivalent associations and configurations involving as many as eight chromosomes. These multiple chromosome configurations in *A. elongatum* indicate extensive chromosome replication with corresponding genic replication.

On the above cytological grounds we may conclude that in the *A. elongatum* crosses there is a numerical preponderance of genes causing awnlessness, and the catalyst controlled by them is more apt to be in excess, with a consequent dominance of awnlessness. In the *A. glaucum* crosses the catalyst would be lower in amount owing to the reduced number of genes causing awnlessness, with consequent intermediacy for the character. Wright's theory applied to our results assumes the accumulative effect of genes in their catalytic activity. There is ample proof for the validity of such an assumption in the work of Stern (4) and Muller, League and Offermann (2) with *Drosophila melanogaster* in which an accumulation of recessive genes brought about an approach to the wild type.

Acknowledgments

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

II. CYTOLOGY OF THE MALE PARENTS AND F_1 GENERATION[1]

BY F. H. PETO[2]

Abstract

Meiosis was studied in *A. glaucum* ($2n = 42$), *A. elongatum* ($2n = 70$), and in the F_1 of these species crossed on varieties of *T. dicoccum, T. durum* and *T. vulgare.* In *A. glaucum* a large proportion of the chromosomes formed bivalents with occasional univalents and quadrivalents. *A. elongatum* was very unusual in that uni-, bi-, tri-, quadri-, quinqui-, sexa-, and octavalent configurations were observed. With one exception the *A. glaucum* × *Triticum* hybrids averaged 4.8–6.2 bivalents per nucleus, thus indicating partial homology between one set of chromosomes from each of the parents. In the *A. elongatum* × *Triticum* hybrids, numerous multivalent configurations were observed and it was concluded that auto- as well as allosyndesis had occurred. Approximately one set of chromosomes remained unpaired in one collection of *T. dicoccum* var. Vernal × *A. elongatum* and approximately two sets remained unpaired in crosses between three varieties of *T. vulgare* and *A. elongatum.*

Two of the crosses exhibited an abnormally small amount of pairing, an effect most plausibly attributed to the reaction of genetic factors limiting prophase pairing.

Tentative conclusions have been made regarding the origin and genetic constitution of *A. elongatum* from the pairing behavior of the chromosomes in this species and its hybrids. It appears likely that *A. elongatum* arose through hybridization between hexaploid and tetraploid species of *Agropyron* with subsequent chromosome doubling. An alternative explanation is also suggested.

Introduction

Studies on the conjugation of the parental chromosomes in hybrids have been particularly valuable in determining the phylogenetic relations between the various species of *Triticum* as well as the relations of these to species of *Aegilops, Secale* and *Haynaldia.* During the summer of 1935, successful crosses were made between certain species of *Triticum* and *Agropyron* (2). Since the material obtained afforded an opportunity of advancing our knowledge of the genetic relation of *Agropyron* to *Triticum,* analyses of chromosome conjugation in these hybrids were made during the past winter.

The conclusions arrived at by this method are based on the assumption that the extent of pairing of the chromosomes at zygotene is conditioned by the degree of genetic homology of the chromosomes involved. Hence, if normal pairing is observed at metaphase of the heterotypic division, it is assumed that there is at least partial genetic homology between the paired chromosomes. Numerous cases have, however, been reported where certain genes may limit or prevent prophase pairing. In consequence, a reduced amount of pairing or a lower chiasma frequency may not always indicate a proportional decrease in genetic homology. This danger of misinterpretation

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may be minimized by studying an adequate number of similar crosses, since it is unlikely that they would all possess the genic complex necessary to suppress normal pairing.

Literature Review

It was reported by Tzitzin (7) that *A. glaucum*, *A. trichophorum*, *A. elongatum* and *A. junceum* crossed readily with various *Triticum* species while no successful crosses were made with any of the other species of *Agropyron*. From a consideration of these facts and the morphological similarities of the above four species to *Triticum* he concluded that these *Agropyron* species should be included in the genus *Triticum*.

Vakar, Krot and Brekina (8) studied the meiotic behavior of the F_1 of *T. durum* × *A. elongatum*, *T. vulgare* × *A. elongatum*, and *T. vulgare* × *A. glaucum*. They found an average of 10 bivalents at the heterotypic metaphase and concluded that conjugation occurred between the sets A and B of *Triticum* with similar sets in *Agropyron*. Sapehin (6) conducted studies on similar hybrids but evidently used a different form of *A. elongatum* since the F_1 of a cross with *T. vulgare* contained 49 instead of 56 chromosomes. Twenty-one bivalents were observed at heterotypic metaphase in this cross. In the cross *T. vulgare* × *A. glaucum* he obtained very different results to Vakar *et al.* since 2–3 bivalents instead of 10 were observed.

Material and Methods

The crosses were made in the Division of Forage Plants at the Central Experimental Farm, Ottawa, during the summer of 1935 and have been reported by Armstrong in the first paper of this series (2). The male parents, *A. glaucum* and *A. elongatum* and nine different crosses with species of *T. dicoccum*, *T. durum* and *T. vulgare* were grown in the greenhouses of the Division of Forage Plants and the National Research Laboratories during the past winter.

Anthers at the proper stage were collected and fixed in absolute alcohol 3 parts and glacial acetic acid 1 part. They were transferred to 75% alcohol after 12–24 hours and stained and made permanent by McClintock's method (4).

Ten, and in some cases twenty, nuclei of each parent and hybrid were analyzed by examination of side views at heterotypic metaphase. Interpretation of polar views was never used since it was found to be impossible to detect and interpret multiple configurations in this manner.

Photomicrographs were used almost entirely for purposes of illustration. It may be impossible in many photomicrographs for the reader to analyze these nuclei completely, nevertheless they should indicate the validity of the author's analysis more accurately than do camera lucida drawings. Two camera lucida drawings are, however, included to supplement the photomicrographs. A Zeiss apo. 1.3 mm. objective and 8 × ocular was used for magnifications below 1000 × while a 25 × ocular was used for photomicro-

graphs of a higher magnification. A green (V.G.3) filter and orange (O.G.1) filter made by Jenaer Glaswerk were used in front of the light source. The pictures were taken on Eastman Panatomic film and developed in D72.

Observations

A. glaucum ($2n = 42$)

Ten metaphase nuclei were completely analyzed and the average frequencies for the various configurations are given in Table I. Four of the nuclei contained 21 bivalents, while of the remainder, five contained two univalents, and one contained four univalents. A typical nucleus is shown in Fig. 1, which contains 19 ring-shaped bivalents with two chiasmata each, one rod-shaped bivalent with one chiasma and two univalents. The chiasma frequency per bivalent in this nucleus is 1.9 which is slightly higher than the average of 1.7. Two hundred tetrads were examined and 4.5% contained micronuclei. It is apparent that only a small proportion of the univalents present behaved abnormally enough to be excluded from the tetrad nuclei.

Two of the metaphase nuclei contained each a chain of four chromosomes, and in other nuclei which were not completely analyzed rings of four chromosomes were seen. This suggests that at some time an interchange of segments between non-homologous chromosomes had occurred.

A. elongatum ($2n = 70$)

Chromosome conjugation in this species is very complex, since multiple configurations are always present. The chromosome number was accurately checked by counts at heterotypic anaphase. Seventy chromosomes can be clearly seen in Fig. 3. Complete nuclei could be analyzed wherever it was possible to find cells in which the contents had been spread out by pressure when the cell was in an oblique position relative to the equatorial plate. Two such nuclei are shown in Figs. 2 and 4. Their constitution is given in the legend. The average frequencies of the various associations for the 10 nuclei analyzed are as follows: 2.0 univalents, 22.1 bivalents, 0.6 trivalents, 3.0 quadrivalents, 0.2 quinquivalents, 0.7 sexavalents and 0.6 octavalents. It is important to note the preponderance of associations with an even valency, the number of quadrivalents being particularly high. Rings of eight chromosomes are of fairly frequent occurrence and in one instance two such rings were observed in a single nucleus. This is believed to be the first time octavalent associations have been observed in a naturally occurring species of Gramineae. A ring of eight at metaphase is shown in Fig. 5 and the same configuration is seen in Fig. 4 at a much lower magnification. An octavalent at diakinesis is shown in Fig. 6 and a diagrammatic interpretation is also included. Further terminalization of such a configuration may form either a ring of eight or a ring of six with an appended pair. The significance of these configurations will be discussed later in relation to the pairing condition found in the hybrids with *Triticum*.

The homotypic division appeared to be relatively normal. Two hundred tetrads were examined and 9% of these had micronuclei. This is twice the percentage found in *A. glaucum*, a result not unexpected considering the complex pairing observed in *A. elongatum*.

Tetraploid Triticum × *A. glaucum* ($2n = 35$)

In the F_1 of *T. dicoccum* var. Vernal × *A. glaucum* there was an average of 20.4 univalents, 6.2 bivalents and 0.8 trivalents per nucleus (Table I) while in the F_1 of *T. durum* var. Mindum × *A. glaucum*, 22.0 univalents, 5.5

TABLE I

CHROMOSOME ASSOCIATIONS IN PARENTAL SPECIES AND F_1 OF *Triticum* × *Agropyron* HYBRIDS

Parent or cross	Chr. No. ($2n$)	No. of cells	Associations of								Configurations	Chrs. associated
			1	2	3	4	5	6	7	8		
A. glaucum	42	10	1.4	19.9		0.2						
A. elongatum	70	10	2.0	22.1	0.6	3.0	0.2	0.7		0.6		
T. dicoccum var. Vernal × *A. glaucum*	35	20	20.4	6.2	0.8						7.0	14.8
T. durum var. Mindum × *A. glaucum*	35	20	22.0	5.5	0.5	0.05					6.1	13.0
T. vulgare var. Lutescens × *A. glaucum*	42	20	30.5	4.8	0.6						5.4	11.4
T. vulgare var. Kharkov × *A. glaucum*	42	20	30.3	5.6	0.01						5.6	11.2
T. vulgare var. C.A.N. 1835 × *A. glaucum*	42	20	39.3	1.2	0.05						1.3	2.6
T. dicoccum var. Vernal × *A. elongatum*												
Collection No. 1	49	7	6.6	12.1	3.6	1.1	0.6				17.4	42.4
Collection No. 2	49	7	17.7	8.3	3.0	0.7	0.6				12.6	31.3
T. vulgare var. Kharkov × *A. elongatum*	56	10	19.5	9.5	3.0	1.6	0.5				14.6	36.9
T. vulgare var. C.A.N. 1835 × *A. elongatum*	56	10	13.0	11.7	3.4	1.7	0.4	0.1			17.3	43.0
T. vulgare var. Lutescens × *A. elongatum*	56	10	14.2	10.6	3.4	1.1	0.8	0.2		0.1	16.2	41.8

bivalents, 0.5 trivalents and 0.05 quadrivalents were present. The presence of approximately 5–6 bivalents indicates partial homology between Set A or B of *Triticum* and one of the sets derived from *A. glaucum*. The occurrence of trivalents would be expected since quadrivalents were occasionally observed in *A. glaucum*. Partial homology would exist, therefore, between certain pairs of chromosomes constituting the gametes of *A. glaucum* and these would form the observed trivalents on pairing with additional homologous chromosomes contributed by the *Triticum* parent.

The chiasma frequency per bivalent for Mindum × *A. glaucum* was only 1.2, while *A. glaucum* had a chiasma frequency of 1.7. The closeness of pairing in the hybrid, therefore, was much weaker than that found in the male parent. In addition there was almost complete termination of the chiasmata in the hybrid since the coefficient was 0.96.

The appearance of the bivalents and trivalents in the Mindum cross can be seen in Fig. 8. This cell was scarcely typical since three trivalents and only two bivalents were present, whereas in other cells the occurrence of trivalents was relatively infrequent as may be seen in Table I. A high degree of pollen sterility would be expected in this cross since more than half of the pollen cells contained micronuclei when examined just prior to the first nuclear division.

Hexaploid Triticum × *A. glaucum* ($2n = 42$)

The meiotic behavior of the F_1 of the varieties Lutescens and Kharkov (*T. vulgare*) crossed with *A. glaucum* was very similar. In the Lutescens cross an average of 30.5 univalents, 4.8 bivalents and 0.6 trivalents were present, while in the Kharkov cross, 30.3 univalents, 5.6 bivalents and 0.01 trivalents were present. Although there were fewer bivalents in the former, this was compensated for by the increased number of trivalents, so that the average number of chromosomes associated in one was 11.4 and the other 11.2. The data in these crosses corroborated, therefore, the conclusion arrived at for tetraploid *Triticum* × *Agropyron* which indicated partial homology between Set A or B of *Triticum* and one of the sets derived from *A. glaucum.* In the hexaploid crosses the presence of a C set of chromosomes accounted for the additional univalents observed.

The chiasma frequency per bivalent in Lutescens × *A. glaucum* was 1.3 and the coefficient of terminalization 0.78, while in Kharkov × *A. glaucum* the chiasma frequency was 1.0 and the coefficient of terminalization 0.94. These differences are of doubtful significance.

Pairing of chromosomes of unequal length was observed in six of the 20 nuclei analyzed in the Lutescens cross. Two heteromorphic bivalents and one heteromorphic trivalent are shown in Text-Fig. 1. These observations indicate that structural changes of considerable magnitude have taken place if these chromosomes have evolved from a common primary set.

The frequency of pairing in the cross C.A.N. 1835 × *A. glaucum* was strikingly different from the above two crosses since an average of only 1.2 bivalents per nucleus was observed. No pairing whatever was found in eight of the twenty nuclei examined. A typical asynaptic nucleus is shown in Fig. 9 in which 42 univalents can be counted at heterotypic metaphase. The largest number of bivalents observed was four.

TEXT-FIG. 1. *Lutescens* × *A. glaucum, one heteromorphic trivalent and two heteromorphic bivalents,* × *2560.*

An average of 1.3 chiasmata per bivalent was observed and these had all completely terminalized. The significance of this abnormally low degree of pairing in this cross will be discussed later.

Tetraploid Triticum × *A. elongatum* ($2n = 49$)

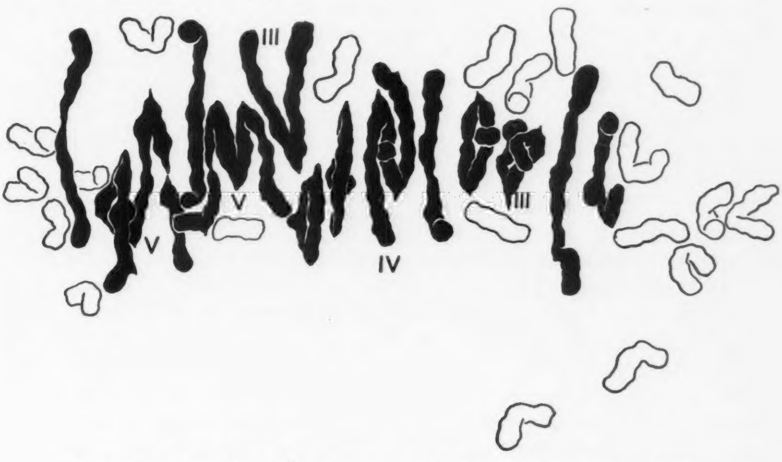
T. dicoccum var. Vernal and *T. durum* var. Mindum were successfully crossed on *A. elongatum*, but only the Vernal × *A. elongatum* cross has been examined cytologically. This cross is particularly interesting in that different collections gave significantly different results. In Collection 1 there were only 6.6 univalents while in Collection 2 there were 17.7 univalents. In the latter there was considerable reduction in the number of bivalents, but the numbers of trivalents, quadrivalents and quinquivalents were very similar in both collections. The identity of individual parent plants was not recorded in these initial crosses, and it is possible that intravarietal differences may exist in the parents. The manner in which such differences could affect the pairing of the chromosomes will be considered in the discussion.

The heterotypic division is not illustrated or described here, since it did not differ from that of other *A. elongatum* crosses, which are described later, except in the frequency of the various configurations. This cross is, however, used to illustrate the behavior in the homotypic division. Fig. 12 shows a typical homotypic metaphase plate from Collection 1. The majority of the chromosomes are undergoing the normal division at the equatorial plate. Single unsplit chromosomes may be seen scattered throughout the cytoplasm and these undoubtedly arose from the univalents which had already split in the heterotypic division. The splitting of univalents in the heterotypic division is shown in Figs. 13 and 15. A homotypic anaphase plate, in which the univalents are no longer distinguishable, is shown in Fig. 11. These univalents nevertheless appear to be responsible for the numerous micronuclei which can be observed in the tetrad stage. A typical tetrad is shown in Fig. 10. The presence of these micronuclei is usually associated with pollen sterility in wide crosses.

Hexaploid Triticum × *A. elongatum* ($2n = 56$)

The F_1 plants of *A. elongatum* crossed with Kharkov, C.A.N. 1835 and Lutescens were available for study. These hybrids had 56 chromosomes, and considerable difficulty was experienced in obtaining metaphase plates in which the chromosomes were separated sufficiently to permit accurate interpretations of the various associations. Ten nuclei of each cross were found in which the whole complement of chromosomes could be resolved. Fig. 14 shows a metaphase plate of C.A.N. 1835 × *A. elongatum*, while Figs. 16 and 17 show plates of Lutescens × *A. elongatum*. The results of the analysis of these plates are given in the legend and the valencies of certain configurations are indicated by numerals on the photomicrographs. While the reader will not be able to interpret all the configurations present in these photomicrographs, he will recognize the possibility of complete analysis of the original preparations by careful study of the configurations at different foci. A

camera-lucida drawing of a nucleus of Kharkov × *A. elongatum* is shown in Text–Fig. 2 in which the configurations have been drawn separately to show clearly the interpretation of configurations of various valencies. The univalents are unblocked, the bivalents are blocked and the multivalent configurations are identified by Roman numerals.



TEXT-FIG. 2. *Kharkov × A. elongatum, 20 × I, 8 × II, 2 × III, 1 × IV, 2 × V, × 1490.*

The univalents split equationally in the heterotypic division and two slightly different anaphase stages are shown in Figs. 13 and 15. In the former, several of the chromosome halves have already become widely separated and it is believed that separation is usually complete prior to cytokinesis. In Fig. 15 the split is just apparent in the lagging univalents. The univalents which have divided equationally in the heterotypic division do not become aligned properly at the equatorial plate at the homotypic metaphase but appear to be scattered throughout the cytoplasm as illustrated for the F_1 of Vernal × *A. elongatum.* Numerous micronuclei are found in the tetrads and young pollen nuclei and consequently a large proportion of sterile pollen might be expected.

It is important to note how closely the frequencies for the various configurations check in these three crosses. The wheat varieties used have widely different origins and their genetical differences might have been expected to be reflected in the relative frequencies of particular configurations. The fact that their behavior is so similar indicates that most of the varietal differences were the result of individual gene changes rather than chromosome aberrations. It should also be noted that these hybrids possess very few quadrivalents (1.1–1.6) but many more trivalents (3–3.4), whereas the situation was reversed in the *A. elongatum* parent where the trivalents were scarce but an average of three quadrivalents per nucleus was observed.

It would not be valid to conclude from the large number of chromosomes associated in these *A. elongatum* crosses, that there was a higher degree of homology between the parental chromosomes involved in these crosses than in the *A. glaucum* crosses where there were relatively few associations. Autosyndesis of the *A. elongatum* chromosomes is undoubtedly responsible for a considerable proportion of the associations observed. The relative proportions of allo- and autosyndesis cannot be determined solely by cytological studies of the hybrids themselves. However, if the genetical constitution and origin of *A. elongatum* can be determined and the evidence from a large number of interspecific and intergeneric crosses in the tribe Hordeae correlated, then it may be possible to estimate the relative proportions of auto- and allosyndesis in these crosses between *Triticum* and *A. elongatum.*

Discussion

There is considerable discrepancy between the observations and conclusions in this investigation and those reported by Vakar, Krot and Brekina (8) and Sapehin (6) on similar material. The former authors concluded that the amount of pairing in the F_1 of *A. elongatum* and *A. glaucum* when crossed with *Triticum* was similar, 10 bivalents on the average being formed. It is inconceivable that these observations can be accurate in view of the results obtained in the present investigation. Careful examination of the camera lucida drawings by Vakar, Krot and Brekina suggests that they have misinterpreted certain meiotic stages. For example their Figs. 6 and 8 do not appear typical heterotypic metaphase plates as named but rather to be in the anaphase stage with univalents splitting at the equatorial plate and with one polar group of chromosomes missing. Further, these authors include one typical side view of metaphase in *T. vulgare* × *A. glaucum* which clearly shows 7 bivalents, in agreement with the writer's observations. However, the majority of their observations were apparently made on polar views where it is extremely difficult to interpret the configurations.

Sapehin (6) evidently used a biotype of *A. elongatum* with 49 chromosomes so that his observations on crosses involving this species are not comparable with those made in the present study. He reports the presence of 2–3 bivalents in the F_1 of *T. vulgare* × *A. glaucum* whereas our cross between Kharkov × *A. glaucum* possessed an average of 5.6 bivalents and C.A.N. 1835 × *A. glaucum* possessed only 1.2 bivalents.

Tzitzin (7) and Vakar, Krot and Brekina (8) concluded that their morphological and cytological evidence indicated that *A. elongatum* and *A. glaucum* should be included in the genus *Triticum* rather than the genus *Agropyron*. These conclusions appear to be scarcely justified at present, since these species do not exhibit a higher degree of homology on crossing with *Triticum* than does *Aegilops* (1). An analogous case is found in *Lolium* and *Festuca,* the relative taxonomic positions of which have not been called in question in spite of the fact that the author (5) has shown that there is complete pairing of the parental complements in the F_1 hybrids of *Lolium perenne* × *Festuca*

pratensis. Cytological demonstration of genetic homology between primary sets of chromosomes or individual chromosomes in wide crosses does not in itself justify changing their taxonomic position. When the genetic relations of the various species of *Agropyron* to one another have been determined through hybridizing and subsequent meiotic studies, and when the relation of the various *Agropyron* species to other members of the tribe has also been determined, it should be possible to arrive at definite conclusions regarding the taxonomic position of *A. elongatum* and *A. glaucum*.

In two of the hybrids studied, the frequency of pairing was much lower than that found in similar crosses or other collections of the same cross. In these instances either the homology of the parental chromosomes was different or else the homology remained unchanged but genes or gene complexes limited the degree of zygotene pairing. Several investigators (*e.g.*, Darlington, (3) have reported cases which indicated a segregation for genes limiting pairing. A similar case was reported in intergeneric hybrids between *Lolium perenne* and *Festuca pratensis* by the author (5) where five plants of a backcross gave chiasma frequencies per bivalent between 1.57 and 1.80 while two plants gave only 0.80 and 0.62. The same explanation would be reasonable for the F_1 of C.A.N. 1835 × *A. glaucum* in which there was an average of only 1.3 configurations per nucleus, whereas similar crosses involving Lutescens and Kharkov gave an average of 5.4 and 5.6 configurations per nucleus. C.A.N. 1835 is, however, a sixth generation hybrid between *T. durum* var. Pentad and *T. vulgare* var. Marquis and consequently a considerable number of structural changes may have altered the chromosomes to such an extent that their pairing behavior in wide crosses would be altered considerably.

The other example of an abnormally low degree of chromosome association was found in Collection 2 of Vernal × *A. elongatum*. In this case the *Triticum* parent was not in itself produced by hybridization. Intravarietal genetical differences in either of the parents would most likely be responsible and differences of this order would more likely be due to gene rather than structural changes. Therefore, at present it appears that this is another instance of genic limitation of pairing. Additional biotypes of these parents will have to be inter-crossed and the pairing behavior of the F_1 observed, before it will be possible to determine definitely the genetical basis for this abnormal behavior.

In spite of the irregularities described above, the normal behavior of the male parents and remaining crosses makes it possible to develop some conception of the genetic constitution and phylogeny of *A. glaucum* and *A. elongatum* and to obtain some information on the genetic relation of *Agropyron* and *Triticum*. This can only be accomplished if the primary sets of chromosomes are dealt with as a unit. Due allowance, however, must be made for the possibility of structural changes altering the pairing behavior of individual chromosomes. This method has already been used with fair success by numerous workers in determining the phylogenetic relation of the genera *Triticum*, *Aegilops*, *Secale* and *Haynaldia* of the tribe *Hordeae* (Aase, 1).

A limited amount of interchange between non-homologous chromosomes in *A. glaucum* has apparently occurred as shown by the presence of quadrivalents in this species and trivalents in the F_1 of crosses with tetraploid and hexaploid species of *Triticum*. The presence of approximately 5–6 bivalents in all but one of the above crosses indicates that either Set A or B of *Triticum* is partially homologous with one of the sets present in *A. glaucum*. Crosses will be made this season between *T. monococcum* and *A. glaucum* to determine which of these two sets is involved. In the meantime to simplify subsequent discussion the homologous set observed will be arbitrarily designated as A. If we designate the other two unidentified *Agropyron* sets as X and Y, then the hypothetical constitution of *A. glaucum* will be AXY.

In *A. elongatum* the situation is very complicated because of the high polyploid condition ($2n = 70$) and the large numbers of multivalent associations observed at meiosis. In spite of the presence of so many multivalent associations, it is unlikely that this species is a true autopolyploid. Numerically it is impossible for this 70-chromosome type to have arisen from repeated doubling of primary sets without hybridization having played some role. It is concluded, therefore, that this species probably arose through crossing of a hexaploid with a tetraploid species which would give rise to an unstable form with 35 chromosomes. The chromosome number in this unstable form would then become doubled to give *A. elongatum* with 70 chromosomes.

The number of univalents, bivalents and multiple configurations found in both *A. elongatum* and its crosses on *Triticum* give us certain clues as to the genetical constitution of *A. elongatum*. A large number of possible constitutions for *A. elongatum* were fitted to the data but only two appear feasible. The first and most promising explanation is that an *A. glaucum*-like hexaploid plant (AXY) was crossed with a tetraploid *Agropyron* of the constitution XY, *viz.*, AXY × XY = AXXYY ($2n = 35$). On doubling of this intermediate form a decaploid species such as *A. elongatum* would result. As pointed out previously, it is a simple matter to check the identity of set A. Additional crosses of *A. elongatum* with other members of the tribe Hordeae of known constitution should demonstrate whether or not X or Y are identical with any of the eight primary sets now identified (Aase, 1).

The pairing expectations from an AXXYY plant on selfing would be 7 bivalents of AA constitution, 7 quadrivalents of XXXX constitution and 7 quadrivalents of YYYY constitution or a total of 7 bivalents and 14 quadrivalents. The actual proportions found differed from the expected by the presence of an excess of bivalents and too few quadrivalents. Fewer quadrivalents may be explained by the fact that competition of pairing frequently results in four homologues forming two bivalents instead of one quadrivalent, and also by the occurrence of interchanges between X and Y which would increase the differentiation between pairs of chromosomes. Interchanges of this kind would also account for the presence of configurations of a higher valency than four. A single exchange of chromatin between X and Y in a plant of the constitution AXXYY could result in a ring of eight being formed and several of these have been observed in *A. elongatum*.

If a plant of the constitution AXXYY were crossed with *T. vulgare* of the known constitution ABC, *viz.*, ABC × AXXYY = AAXXYYBC ($2n$ = 56), 14 univalents and 21 bivalents would be expected. Comparing this with the reported frequencies for the *T. vulgare* crosses in Table I, it will be seen that the agreement for univalents is satisfactory while the actual number of bivalents is much less than expected and there is a proportional excess of configurations of a higher valency. While differentiation between pairs within four homologues in the *A. elongatum* parent would reduce the number of quadrivalents observed, because of competition of pairing, the same factors which caused this differentiation (*i.e.*, interchange between X and Y) would be expected to increase the number of multiple configurations in the F_1 of hybrids with *Triticum*. Since there is ample evidence that interchange has occurred frequently, the apparent discrepancies between the expected and observed number of configurations would be satisfactorily explained. The striking similarity between the numbers of trivalents, quadrivalents and quinquivalents observed in the crosses with different varieties of *T. vulgare* indicates that the structural differentiation involved was confined to the *A. elongatum* parent.

If a plant of the constitution AXXYY were crossed with *T. dicoccum* (AB), seven univalents would be expected and this agrees well with the observed frequency of 6.6 for Collection 1 of Vernal × *A. elongatum*. The number of bivalents and configurations of a higher valency are similar to those observed in the *T. vulgare* crosses and their occurrence can be explained in the same manner.

The alternative explanation of the constitution of *A. elongatum* is that it arose through the crossing of a hexaploid *Agropyron* species of the constitution ABY with a tetraploid wheat of the constitution AB, *viz.*, AB × ABY = AABBY ($2n$ = 35). On doubling of this intermediate form, a decaploid species would result. The latter, on selfing, should form 7 bivalents and 14 quadrivalents, which is similar to the expectations for the alternative constitution AXXYY.

If a plant of the constitution AABBY was crossed with *T. vulgare* ABC, *viz.*, ABC × AABBY = AAABBBCY ($2n$ = 56), 14 univalents and 14 trivalents would be expected. Again we find a close approximation between the expected and observed number of univalents. However, there are present less than one-third of the expected number of trivalents with all the bivalents and multiple configurations unaccounted for. In triploid species it is common to find only two of the three homologues pairing. If this occurred in these hybrids then the observed number of univalents would be expected to be much larger than observed. The occurrence of multiple configurations higher than three would again be accounted for by occasional interchanges. A partial test of the relative validity of these alternatives will be afforded this season by crossing *A. glaucum* with *A. elongatum*. If *A. elongatum* is of the constitution AXXYY, then no univalents should be present in the F_1; however, if its constitution is AABBY, 7 univalents should be present.

The first explanation of the origin and constitution of *A. elongatum* is the more attractive for the following reasons: (1) An interspecific cross is more likely to occur in nature than an intergeneric cross. (2) It seems unlikely that a hexaploid *Agropyron* species already exists with two wheat-like sets of chromosomes A and B. (3) From the morphological similarities of *A. glaucum* and *A. elongatum*, it is not unreasonable to suppose that one arose from the other through hybridization and subsequent chromosome doubling.

Acknowledgments

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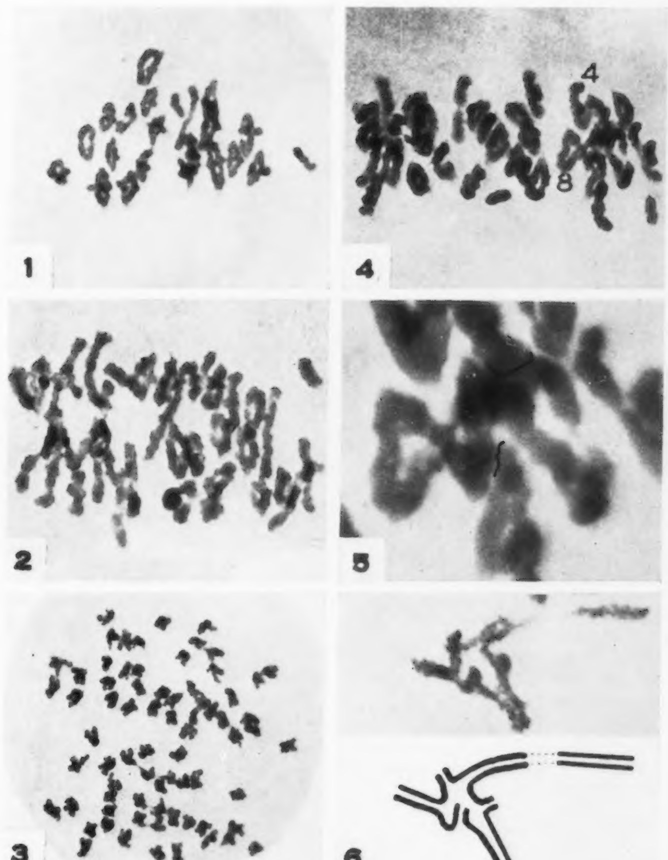

FIG. 1. *A. glaucum*, *2 × I, 20 × II, × 1000.* FIG. 2. *A. elongatum, 2 × I, 19 × II, 6 × IV, 1 × VI, × 1000.* FIG. 3. *A. elongatum, heterotypic anaphase, 70 chr., × 650.* FIG. 4. *A. elongatum, 2 × I, 22 × II, 4 × IV, 1 × VIII, × 650.* FIG. 5. *A. elongatum, octavalent from Fig. 4, × 1790.* FIG. 6. *A. elongatum, diakinesis, octavalent and diagram showing interpretation. × 1140.*

NOTE.—*Roman numerals indicate valency of configuration, thus 2 × I = 2 univalents, etc.*

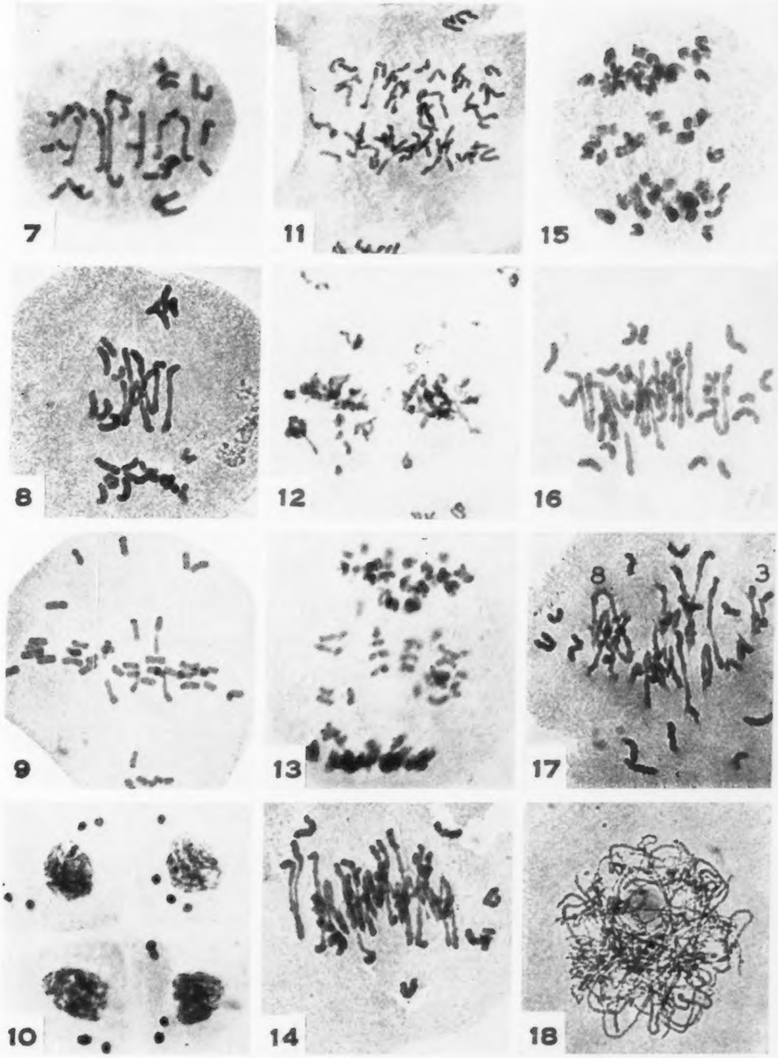

FIG. 7. *Lutescens* × *A. glaucum, metaphase, 28 × I, 7 × II.* FIG. 8. *Mindum* × *A. glaucum, metaphase, 29 × I, 2 × II, 3 × III.* FIG. 9. *C.A.N. 1835* × *A. glaucum, metaphase, 42 × I.* FIG. 10. *Emmer* × *A. elongatum, tetrad containing micronuclei.* FIG. 11. *Emmer* × *A. elongatum, homotypic anaphase.* FIG. 12. *Emmer* × *A. elongatum, homotypic metaphase.* FIG. 13. *Kharkov* × *A. elongatum, heterotypic anaphase, univalents splitting.* FIG. 14. *C.A.N. 1835* × *A. elongatum, 10 × I, 10 × II, 4 × III, 1 × IV, 2 × V.* FIG. 15. *C.A.N. 1835* × *A. elongatum, heterotypic anaphase, 12 univalents splitting.* FIG. 16. *Lutescens* × *A. elongatum, 15 × I, 10 × II, 1 × III, 2 × IV, 2 × V.* FIG. 17. *Lutescens* × *A. elongatum, 13 × I, 10 × II, 5 × III, 1 × VIII.* FIG. 18. *Lutescens* × *A. elongatum, leptotene stage. Magnifications of Figs. 7–18, × 650.*

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SPERMATOCYTE CHIASMA FREQUENCY IN STRAINS OF MICE DIFFERING IN SUSCEPTIBILITY OR RESISTANCE TO THE SPONTANEOUS OCCURRENCE OF MALIGNANT TUMORS[1]

By C. Leonard Huskins[2] and E. Marie Hearne[3]

Abstract

Significant differences have been found in the spermatocyte chiasma frequencies of ten strains of mice differing in genetic susceptibility or resistance to the spontaneous occurrence of malignant tumors. In three resistant and five susceptible strains there is a positive correlation between susceptibility and low chiasma frequency, and between resistance and high frequency. Two inter-related strains may be exceptional, but their cancer and leukemia records are not clearly established, and one of them, though classed as resistant, is somewhat anomalous genetically.

The study comprises a total of 16 tests, each made on 25 complete cells. It was made as an indirect test of a working hypothesis on the mechanism of genetic susceptibility. This hypothesis is based on: (a) the assumed validity of the somatic cell mutation theory of cancer in its most general formulation, (b) the conclusion that gross chromosome irregularities commonly found in tumor cells cannot in themselves be the cause of cancer, though they may be a direct result of some less obvious lack of correlation in the mitotic process, (c) the fact that in different organisms the existence of genes controlling chromosome structure and behavior and other aspects of cell division has been established, (d) the observation that in tumors themselves the chromosomes are commonly found to be "split" precociously, and (e) the observation that "splitting" of meiotic chromosomes is correlated with the absence of chiasma formation. The hypothesis postulated that in strains of mice genetically susceptible to the spontaneous occurrence of tumors, though not yet having cancer, the chromosomes might be found to be "split" slightly in advance of the normal period, and that this could be measured indirectly, but most precisely, by its anticipated effect of causing lowered chiasma frequency at meiosis. The precocious "splitting" would itself be a primary result of some genic difference in the constitution of susceptible animals and might either—through causing grosser chromosome irregularities—lead directly to neoplastic growth, or be a primary morphological concomitant of an underlying cause common to both phenomena.

Introduction

The idea that chromosome aberrations, which are so frequently observed in tumors, are themselves the cause of tumor development has been very widely held since it was first mooted in 1891 by von Hansemann (25) and shortly afterwards developed as a general theory by Boveri (10, 11). Winge (55, 56) and others have stressed the causal significance of polyploidy—the multiplication of whole chromosome sets—rather than aberrations involving irregular chromosome numbers. On the other hand, it has been as widely argued that

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both of these types of nuclear change are the result of the conditions under which tumors develop, and not the cause of their development. Andres (2) may be cited as one of a number of workers who have shown clearly that none of the major aberrations of mitosis are specific for cancer and that in both primary tumors and metastases there may be normal chromosome number and form.

Chimaeras of plants or animals provide abundant evidence that most cytological aberrations do not cause cancer. In chimaeras generally there is a tendency for the patches of tissue made up of aberrant cells to be crowded out by normal cells, and Gowen (23) has pointed out that the aberrant tissues in *Drosophila* gynandromorphs do not show uncontrolled growth.

Seecof (52) showed that in a new transplantable tumor of the rat, giant (polyploid) cells first appeared in the eighth transplant generation; that the largest number of giant cells occurred in tumors that grew rapidly at first and then stopped; that transplantations rarely "take" after this, and that if they do "take" they show no giant cells in the transplants. This, with other studies, seems to make a fairly conclusive case against the polyploidy hypothesis of cancer causation. In crown gall of plants, polyploid cells may be of significance, but, as Levine (34) points out, this is a "protective and reparative process" whereas, he concludes, "Cancer is a disease of the cell which manifests itself in division."

There is, however, one cytological peculiarity of tumor cells which might, *a priori*, be expected to have some more general relationship to the cause of cancer. This is the precocious splitting of some chromosomes, or, more properly (since it is now clear that chromosomes may split one or more division cycles in advance of that in which their separation occurs, (*cf.* 28)), the precocious separation of daughter half-chromosomes before metaphase which was first emphasized by Ludford (39, 40). He observed it in the Jensen Rat Sarcoma, Mouse Sarcoma 375, Fibiger's Tar Sarcoma and Mammary Carcinoma 63. It was the observation by the senior author in 1929 of this precocious "split" in Dr. Ludford's preparations that led eventually to the formulation of the working hypothesis to test which the studies here reported were undertaken. Similar precocious "splitting" of the chromosomes, besides tetraploidy and other aberrations, was also seen in preparations of the Walker Rat Sarcoma kindly demonstrated to the senior author by Dr. Margaret Lewis in 1931. Precocious "splitting" was found by Lewis (35) in tissue cultures of several spontaneous tumors of the mouse also but at that time she was inclined to consider malignancy as bound up with some permanent alteration of the centrosome apparatus, and the chromosome abnormalities as secondary (36). Lately, Lewis and Strong (37) have demonstrated precocious chromosome "splitting" in tissue cultures of 51 spontaneous tumors, all carcinomas of the breast, from seven strains of mice. Of these they state, p. 86, "The growths from the 51 tumours taken from seven strains of mice exhibited chromosomes that were so widely split during prophase and metaphase that they often appeared as pairs of chromosomes of the same shape and size. The longitudinal division of the chromosomes was sometimes

evident in the early prophase, usually in the late prophase, and practically always in the metaphase. At the beginning of anaphase the halves of the chromosomes became separated, one half going to each anaphase plate. The split condition of the chromosomes was evident in mitotic figures in which the number of chromosomes was increased, as well as in those that had the normal number (Figs. 27 and 34). The chromosomes that lagged behind during the migration to form the metaphase were split (Fig. 36), but those that lagged in the separation to form the anaphase, telophase, and daughter nuclei were usually separated and appeared singly rather than split (Fig. 39)."

They comment later on the uniformity of the general cell type in these spontaneous mouse tumors and emphasize that the results are different from what they have found in tissue cultures of human tumors and of the spontaneous tumors of Dr. Walker's inbred rats. In the discussion section of this paper written by Dr. Strong he states: "Thus from the study of tissue cultures it is evident that peculiarities of chromosome behaviour may be not fundamental but only coincident or the result of the original production of certain types of cancerous tissue. Thus the intrinsic factors, such as must be assumed to explain the findings of transplantation, may also be called upon to explain the retention of tissue specificity found to persist in tissue cultures."

Potter and Richter (50) found precocious separation of chromosome halves to be very marked in the mitotic divisions of lymphocytes of mice affected with lymphatic leukemia.

Though precocious chromosome "splitting" may be a general characteristic of malignant cells, so long as it is observed only in the malignant cells themselves there remains no way of deciding whether it is causal or resultant.

On the other hand, should any degree of precocious separation be found in normal cells of animals highly susceptible to the occurrence of spontaneous tumors, and not in resistant strains, it would clearly indicate that precocious separation is either directly causal or primarily associated with the cause of malignancy. The fact that genetically pure strains of mice differing greatly in their degree of susceptibility to spontaneous tumor formation now exist, and the readiness of Dr. C. C. Little of the Roscoe B. Jackson Memorial Laboratory to supply other workers with many of these strains, made it possible for us to examine this aspect of the cancer problem.

Before proceeding to a report of the data obtained, it may, however, be well to summarize briefly other evidence which indicates either directly or by analogy that malignancy arises through a change in the nature of individual cells, and that such changes are of a genetic nature, since these are the primary postulates underlying our study.

The somatic cell mutation theory of cancer (using the term "mutation" in its widest sense as implying some undefined type of permanent change in the hereditary material) appears to have been gaining ground steadily from the time of Ross (51), Oertel (48), Bauer (4) on to the current work of Curtis.

Dunning and Bullock (15–17), which appears particularly conclusive. Very complete reviews of the evidence available up to their time of publication have been presented by Ludford (40) and Levine (34). Some direct, though not conclusive, evidence is provided by Mottram's (43) experiments in inoculating mice with X-rayed testicular tissue, in the course of which one tumor, apparently caused by an X-ray mutation, was produced. In their work on rats with *Cysticercus fasciolaris*, the larval form of *Taenia taeniaeformis* which is the common tape worm of the cat, Curtis, Dunning, and Bullock (16) have shown that though the host may have from one to one hundred benign cysts, malignancy is usually confined to a single cyst. "Of 4,321 cysticercus tumours, 79.3% were sarcomata of the large-cell type and probably arose from the cells which formed the inner zone of the cyst wall, 20.6% were sarcomata composed of smaller cells and possibly arose from the outer zone of the cyst wall, 6 were adenomata, and one was a carcinosarcoma which must have arisen from the snared off bile duct and liver epithelium which was embedded in the wall of the cyst." They conclude that "If the change of a normal to a tumour cell results from a process analogous to somatic mutation which is brought about by the chance action of an irritant it would be expected that the types of cysticercus tumours observed would represent the various types of cells found in the cyst wall and that each would be represented by a number proportional to the comparative number of these cells exposed to the irritant and their relative proximity to it. The above classification [see their Table IX] shows that they roughly approximated this expectation."

Evidence from analogy for the genetic nature of malignancy is provided by the occurrence of a gene in *Drosophila melanogaster* which produces tumors; the occurrence of melanotic neoplasms in intergeneric hybrids of *Platypoecilus* and *Xiphophorus* (21); of tumors on interspecific hybrids of birds (Poll, 1920, cited by Kostoff (32)); of *Xeroderma pigmentosa* produced by a recessive gene in human beings; and of tumors resembling crown gall tumors on interspecific hybrids of *Nicotiana glauca* and *N. langsdorfii* (32, 54). Jones (31) has pointed out some possible analogies with chromosome deficiencies in maize and other organisms. Mottram (44) has obtained irregularities of mitosis as a result of treatments with tar and gentian violet.

The now fairly long list of cases known in which genes affect either the mitotic or meiotic processes and the mechanism of cell division provides further evidence *by analogy* that malignancy may be due to mutation. Amongst these may be cited the recessive gene causing long meiotic chromosomes in *Matthiola* (33) which has other correlated effects also (3, 49). In *Drosophila melanogaster* there are genes affecting crossing over and somatic elimination of chromosomes (12, 22, 53, and others). In maize, Beadle (5–7) has found different genes causing asynapsis, failure of cytokinesis, and polymitosis. Asynapsis caused either by genes or chromosome deficiencies is known also in at least a dozen other plants (see 26 for some of these). In *Allium*, interstitial localization of chiasmata in chromosome pairing is genetically deter-

mined, probably by a single recessive factor (20). It is thus clearly demonstrated that the chromosomes carry genes necessary for self-regulation during karyokinesis, and there is therefore, by analogy, a strong *a priori* case for the assumption that loss of regulatory genes may cause malignancy.

Materials, Methods, and Observations

Observations were originally made upon spermatogonial cells of male mice four to five weeks old, for the reason that these are among the largest and easiest of mammalian somatic cells in which to study mitosis and chromosome form and behavior. It has since appeared that for our purpose of studying the degree of chromosome separation before metaphase, cells in which the chromosomes are less contracted may be preferable. With the fixing and staining technique here described, and various modifications of it, lymph-gland leucocytes have been found to provide better preparations for the detailed study of mouse chromosome shape than any other tissue or method yet tried; other difficulties, however, arise. In the spermatogonial cells here reported on, separation of chromosome halves appeared often to be slightly precocious in strains of mice highly susceptible to the spontaneous occurrence of tumors, but the difference, if any, between susceptible and resistant strains was too slight and too variable to afford any conclusive evidence.

An indirect test of possible differences in the time of somatic chromosome splitting or separation was therefore made, on the hypothesis that precocity of splitting is correlated with a reduction in the tightness of chromosome pairing during meiosis or, in current cytological terminology, with a reduction in frequency of chiasma formation during the meiotic prophase. Data obtained from asynaptic wheat and oats (26) and from *Fritillaria meleagris* (29) provided the basis for this subsidiary hypothesis. Whatever the merits of this hypothesis, the data obtained in applying it to the strains of mice studied appear to have significance for the cancer problem.

The description of the strains used, as given in the printed leaflet provided by the Roscoe B. Jackson Memorial Laboratory, is as follows.

Strain A. Albino mice in which there is a high incidence of spontaneous mammary tumors among breeding females at an average age of nine to twelve months. Inbred since 1918.

Strain D. Dilute brown mice differing from wild mice by three mendelian characteristics, a strain high in the incidence of spontaneous mammary tumors at an average age in breeding females of 10½ months. Inbred since 1909.

Strain C 57 Black. Differing from wild mice by one mendelian recessive characteristic. A high fertility strain in which, during the last ten years there has been no record incidence of carcinoma of the breast. Inbred since 1921.

Strain M Leaden. Mice differing from wild stock by three mendelian recessive characteristics. A high fertility strain with extraordinarily low cancer incidence. Inbred since 1921.

Strain Y. Yellow or brown mice with good fertility, a physiological tendency to a high degree of adiposity and a relatively high occurrence of spontaneous sarcoma of different types. Inbred since 1925.

Strain C-3-H. Colored like the wild house mice. High fertility and high incidence of spontaneous mammary tumors. Average age of tumor incidence eleven months. Inbred since 1921.

Strain CBA. Wild type color. High fertility. Have been selected toward longevity for fourteen generations. No tumors of any descriptoin in individuals whose average age of life for the past ten generations is twenty-one months. Inbred since 1921.

Strain N. Four recessive factors, dilute, brown, non-agouti, piebald. No tumors observed for ten generations. Average age at death is eighteen months. Inbred since 1921.

Strain I. Carry five recessive factors, the same as in strain "N" with pink-eye additional. No tumors have been observed in twelve generations. Inbred since 1921.

Strain C 57 Brown. Have tumors at an advanced age, medium fertility. Inbred since 1921.

The "susceptible" strain designated "Carrel's C 57 Black" in the present study was likewise obtained from Dr. Little who wrote (January 11, 1935) concerning it: "I am accepting the statement of its susceptibility from Alexis Carrel of the Rockefeller Institute. None of the mice of this stock in this laboratory have reached an age when first-hand information is available."

Various fixatives were used in preliminary studies. The most satisfactory method of preparation, and the one used exclusively after the first five strains were examined, was to kill the animals by decapitation, quickly remove one of the testes, cut it into three pieces, smear each *immediately* (speed is essential) on a clean glass slide and plunge this face downwards (in a dish with two ribs on the bottom to prevent the smear touching it) into Kaiser's solution: 10 gm. bichloride of mercury; 3 cc. glacial acetic acid; 300 cc. distilled water. This was then repeated as quickly as possible with the other testis. After about two hours in Kaiser's solution the slides were transferred to Navashin's fixative and kept in it overnight (8, p. 244). They were then stained by a modified iodine crystal-violet method: after washing in tap water, average pH 7.5, the slides were stained for 15 minutes in 1% aqueous crystal violet. They were then rinsed in tap water and plunged into 95% ethyl alcohol for about 5 seconds. From this they were transferred to an 80% alcohol, 1% iodine, and 1% potassium iodide solution for about 45 seconds, and then passed slowly through 95% and absolute alcohol into a mixture of absolute alcohol and clove oil, equal parts. They were then differentiated under the microscope and washed for at least one hour in four changes of xylol and mounted in Canada balsam.

Observations were begun on Strain A (susceptible) from which chiasma frequency counts were made on 15 primary spermatocyte cells. Fifteen were then examined from strain CBA (resistant), then 10 from A, followed by a further 10 from CBA (see 27). In these first two strains the counts were made first independently by both authors and then jointly, and in cases where any doubt existed the most probable number was decided on. For later strains the maximum and minimum (within the standard used) were recorded for doubtful bivalents. Calculations of significance made by using alternately the higher and lower values for successive nuclei were not appreciably different from those presented, in which minimum values have been used throughout. Apart from the maxima and minima of doubtful cases the standard used throughout was that of "minimum chiasma frequency". That is to say, each bivalent configuration was assessed as having the minimum number necessary for the formation of such a configuration. Certain bivalents have quite certainly had more chiasmata than are assessed, but the arbitrary minimum standard is the only one that can be maintained successfully. Since different workers may have different standards, or may prefer to count

the chiasmata at different stages of meiosis, it is only the relative value for different strains evaluated by the same standards which are significant, and not the absolute values per nucleus. This has already become obvious from disagreements between different cytologists working on maize. It is also to be expected, and there is some supporting evidence, that chiasma frequency will differ with the age of the animals (see also Crew and Koller (14)) and with temperature or other environmental influences, as does its genetic correlative, or partial correlative, the crossing-over frequency. The frequencies reported probably do not, however, represent the initial formation frequency and adequate chiasma counts cannot, by present methods, be made at the earliest diplotene stages in mice. For the Wistar Rat, Bryden (13) gives terminalization coefficients of 0.475, 0.503, and 0.577 for ages 2, 8, and 17 months, which he considers "point to a difference in the original frequency of chiasma formation" at these ages.

It should be added further that the term chiasma is here used in the sense understood by Darlington (18) and his school in which all points of intimate contact between paired homologous chromosomes in meiosis are judged to be either "interstitial chiasmata" or "terminalized chiasmata" according to their position. Actually, following studies of *Trillium* (30) the senior author has come to agree with Belling, Metz, and others that terminal junctions are *not necessarily* chiasmata or the result of chiasmata. In a strict sense we now describe as "chiasmata" only those junctions, either terminal or interstitial, between homologous chromosomes at which all four chromatids of the bivalent can be seen and at which two of the chromatids exchange partners. However, pending the acquisition of further knowledge regarding limitations of the chiasma theory of pairing, it is simplest to call all points of contact chiasmata, even though our present viewpoint prevents us attributing significance to terminalization coefficients etc. that might be derived from these data. In the present study only those cells in which the chiasma frequency of every bivalent can be determined have been considered. Since previous studies of chiasma frequency in the mouse have been made on incomplete cells in sectioned material the results are not significantly comparable. Where chiasmata were counted at widely different stages of diakinesis in the earlier part of this study, it was of course necessary, on account of reduction through terminalization, to assume that equivalent numbers of cells at the various stages were included in the totals compared. The later counts were all made at mid to late diakinesis.

In Table I, the data on number of animals, their ages, and the fixatives used are presented.

During the course of this study a strong general impression has been gained that both in mitosis and meiosis the high-chiasma-frequency strains have their chromosomes less contracted than those of low-frequency strains at comparable stages. It has not, however, been possible to obtain significant comparable measurements.

FIG. 1. *Primary spermatocyte chromosomes at early and late diakinesis stages. Each row illustrates the complement of a single nucleus; the total "chiasma frequency" is given in brackets as follows: 1(36), 2(35), 3(31), 4(25), 5(32), 6(30), 7(34), 8(20), 9(32), 10(30). All drawings made at 5800—, reduced to 3700× in reproduction.*

1 : Strain CBA, early diakinesis 2 : Strain CBA, late diakinesis; 3 : Strain A, early diakinesis; 4 : Strain A, late diakinesis; 5 : Strain Y, yellow, early diakinesis; 6 : Strain Y, yellow, late diakinesis; 7 : Strain Y, black, early diakinesis; 8 : Strain Y, black, late diakinesis; 9 : Strain C57 Black, early diakinesis; 10 : Strain C57 Black, late diakinesis; 11–14 : Spermatogonial mitotic metaphases of Strains, A, CBA, C57 Black, and Y, black, respectively.

TABLE I

RECORD OF ANIMALS USED FOR CHIASMA FREQUENCY TEST

Strain	No. of Mice	Approx. ages in months	Fixative
A, First Test	4	1, 1, 4, 4	(a) 2 BE 2 hours; Navashin overnight (b) 2 BD 2 hours; Navashin overnight
CBA, First Test	6	1, 1, 2, 2, 2½, 4	(a) Navashin overnight (b) 2 BE 2 hours; Navashin overnight
Y Black	2	2, 2	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
Y Yellow	1	2½	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
C 57 Black, 1st Test	2	1, 1	(a) 2 BE overnight (b) 2 BE 2 hours; Navashin overnight
I	3	2, 2, 2	Kaiser's 2 hours; Navashin overnight
N	4	2, 2, 2, 2	Kaiser's 2 hours; Navashin overnight
A, Second Test	1	4	Kaiser's 2 hours; Navashin overnight
D	2	1, 1	Kaiser's 2 hours; Navashin overnight
M Leaden, 1st Test	1	1	Kaiser's 2 hours; Navashin overnight
C-3-H	2	1, 1	Kaiser's 2 hours; Navashin overnight
C 57 Brown	2	1, 1	Kaiser's 2 hours; Navashin overnight
Carrel's C 57 Black	2	1, 1	Kaiser's 2 hours; Navashin overnight
C 57 Black, 2nd Test	1	8	Kaiser's 2 hours; Navashin overnight
CBA, 2nd Test	1	8	Kaiser's 2 hours; Navashin overnight
M Leaden, 2nd Test	1	2	Kaiser's 2 hours; Navashin overnight

In Table II the data obtained from 16 distinct studies of "chiasma frequency", each made on 25 cells in 11 different strains of mice, are presented.

It will be noted that two studies each have been made on CBA, C 57 Black, A, Y, and M Leaden. As mentioned, A and CBA were the first two strains examined. The second set of observations on these strains was made about a year after the first set in order to check any possible change produced by changes in technique or increased facility in observation or, in the case of CBA, by age. Strains C 57 Black and M Leaden were double-checked because they are reported to be resistant and their chiasma frequency was found to be low like that of the susceptible strains. Strain Y is heterozygous for A^y since this gene is lethal when homozygous. Little (38) has shown a significantly higher incidence of mammary tumors in its non-yellow segregates, but not of other types of tumors. The yellow segregates have a chiasma frequency 0.84 higher than that of the blacks; this, though below the standard of significance we have selected, is in accord with the difference in tumor incidence.

TABLE II

	N	I	CBA, first test	CBA, second test	C 57 Black, first test	C 57 Black, second test	Y Yellow	Y Black	Carrel's C 57 Black	C-3-H	D	C 57 Brown	A, first test	A, second test	M Leaden, first test	M Leaden, second test
	34	33	36	32	31	34	31	33	26	28	26	26	27	26	29	26
	33	35	32	29	33	29	31	28	28	29	24	30	31	30	28	28
	36	33	31	33	30	28	29	30	25	28	25	26	32	27	23	27
	36	35	33	33	33	28	29	31	25	30	25	29	25	31	29	29
	37	34	34	31	28	27	27	31	28	29	25	29	33	30	31	30
	38	36	34	34	33	34	31	33	31	32	31	26	29	32	26	27
	38	37	32	32	28	28	29	31	25	30	29	28	32	29	28	29
	34	34	35	32	29	30	30	28	29	30	30	30	29	28	32	27
	34	34	34	32	29	28	33	31	26	30	29	26	31	31	28	28
	33	35	33	32	31	31	25	26	25	31	28	28	29	29	27	30
	33	34	36	32	28	31	28	28	26	28	26	28	30	30	27	25
	36	31	33	35	30	29	29	29	26	27	28	26	29	27	24	26
	33	32	33	29	29	29	32	32	24	27	28	26	25	30	32	25
	36	32	31	30	29	30	32	30	26	27	26	29	25	30	29	28
	32	36	32	27	28	29	31	26	27	30	27	28	31	28	25	24
	38	32	32	30	31	33	32	26	25	29	28	28	32	28	29	24
	37	33	33	30	32	31	29	29	28	28	28	25	28	29	29	25
	33	31	35	34	29	32	32	27	27	32	27	27	29	31	30	24
	34	35	30	32	30	28	30	29	26	26	29	29	27	30	28	25
	35	33	29	31	31	26	31	30	26	29	28	29	28	30	27	30
	34	36	31	35	33	29	29	27	26	32	31	29	26	31	25	26
	38	33	31	34	31	28	29	32	28	33	25	27	28	29	28	28
	31	35	30	35	31	30	29	28	29	30	27	28	25	28	26	27
	34	34	34	30	29	31	31	27	28	28	26	32	26	29	27	25
	36	35	31	29	32	30	31	27	28	26	29	28	24	28	27	24
Sum	873	848	815	793	758	743	750	729	668	729	685	697	711	731	694	667
Mean	34.92	33.92	32.60	31.72	30.32	29.72	30.00	29.16	26.72	29.16	27.40	27.88	28.44	29.24	27.76	26.68
Sum χ^2	30585	28826	26653	25263	23052	22183	22578	21373	17914	21345	18857	19497	20387	21427	19386	17891

Grand Total = 11891 *Total sum of (variates)^2 of all columns = 357217* *Necessary difference for P = 0.01 = 1.40.*

The data have been analyzed by Fisher's method for the Analysis of Variance, and P = 0.01, which requires a difference of 1.4, has been selected for the estimate of significance; in view of the difficulty of obtaining absolutely accurate counts, even the significance of 99 : 1 odds cannot, we consider, be taken too literally. The differences are set forth in Table III.

Discussion

It is apparent from Table III that all the strains listed by the Roscoe B. Jackson Laboratory as having a high incidence of spontaneous tumors have also a low chiasma frequency. Strains A, D, and C-3-H, have "a high incidence of spontaneous mammary tumors", Strain Y has "a relatively high occurrence of spontaneous sarcomata of different types", and Strain C 57 Brown "has tumors at an advanced age." Their chiasma frequencies are: 28.4 and 29.2 in the two tests of Strain A, 27.4 in D, 29.2 in C-3-H, 30.0 in Y yellow, 29.2 in Y black and 27.9 in C 57 Brown. Carrel's C 57 Black which is also, unofficially, reported as susceptible has likewise a very low chiasma frequency—26.7.

The highest chiasma frequencies, 34.9, 33.9, and 32.6, occur in strains N, I, and CBA, which are respectively described as having "no tumors observed for 10 generations"; "no tumors . . . . . . observed in 12 generations"; and "no tumors of any description in individuals whose average length of life for the past 10 generations is 21 months." This latter strain has been "selected toward longevity for 14 generations." In these nine strains there is, then, a correlation between susceptibility to spontaneous tumor formation and low chiasma frequency, on the one hand, and, on the other, between high chiasma frequency and immunity (so far as records are available) from spontaneous tumor formation.

In two other strains reported as resistant, the chiasma frequency is, however, low and the correlation does not hold. At the same time it appears that the genetic and tumor-incidence characteristics of these strains may possibly also be partly anomalous. With the kind permission of Dr. C. C. Little and others concerned some unpublished observations on these strains are therefore presented though not with any intention of "explaining away" our exceptions. Until further detailed analyses of the cancer record of these strains are completed and published it will obviously be impossible to decide whether or not they constitute real exceptions to the apparent rule found in other strains, of low chiasma frequency being associated with susceptibility and high with resistance.

Strain C 57 Black, the fifth strain to be examined, was the first strain reported as resistant which was found to have a low chiasma frequency. It is described as having "no recorded incidence of carcinoma of the breast . . . . . . during the last ten years." After its chiasma frequency had been found to be somewhat low (30.3) in the first test, this result was communicated to Dr. Little with a request for further information regarding the possible occurrence of other types of tumors in this strain. In reply Dr. Little stated

TABLE III

DIFFERENCES IN CHIASMA FREQUENCY IN STRAINS OF MICE

Necessary difference for P = 0.01 = 1.40

	CBA, 2nd test 31.72	CBA, 1st test 32.60	I, 33.92	N, 34.92	C 57 Black, 2nd test 29.72	C 57 Black, 1st test 30.32	M Leaden, 2nd test 26.68	M Leaden, 1st test 27.76	Carrel's, C 57 Black, 26.72	C 57 Brown, 27.88	Y Black, 29.16	Y Yellow, 30.00	D, 27.40	C-3-H, 29.16	A, 2nd test 29.24	A, 1st test, 28.44
A, 1st Test	3.28	4.16	5.48	6.48	1.28	1.88	1.76	.68	1.72	.56	.72	1.56	1.04	.72	.80	—
A, 2nd Test	2.48	3.36	4.68	5.68	.48	1.08	2.56	1.48	2.52	1.36	.08	.76	1.84	.08	—	—
C-3-H	2.56	3.44	4.76	5.76	.56	1.16	2.48	1.40	2.44	1.28	0.00	.84	1.76	—	—	—
D	4.32	5.20	6.52	7.52	2.32	2.92	.72	.36	.68	.48	1.76	2.6	—	—	—	—
Y Yellow	1.72	2.60	3.92	4.92	.28	.32	3.32	2.24	3.28	2.12	.84	—	—	—	—	—
Y Black	2.56	3.44	4.76	5.76	.56	1.16	2.48	1.40	2.44	1.28	—	—	—	—	—	—
C 57 Brown	3.84	4.72	6.04	7.04	1.84	2.44	1.20	.12	1.16	—	—	—	—	—	—	—
Carrel C 57 Black	5.00	5.88	7.20	8.20	3.00	3.60	.04	1.04	—	—	—	—	—	—	—	—
M, 1st Test	3.96	4.84	6.16	7.16	1.96	2.56	1.08	—	—	—	—	—	—	—	—	—
M, 2nd Test	5.04	5.92	7.24	8.24	3.04	3.64	—	—	—	—	—	—	—	—	—	—
C 57 Black, 1st Test	1.40	2.28	3.60	4.60	.60	—	—	—	—	—	—	—	—	—	—	—
C 57 Black, 2nd Test	2.00	2.88	4.20	5.20	—	—	—	—	—	—	—	—	—	—	—	—
N	3.20	2.32	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—
I	2.20	1.32	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CBA, 1st Test	.88	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CBA, 2nd Test	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

that only one malignant tumor, some sort of lymphoblastoma, had been reported in it at Bar Harbor during the last 10 years, but that Dr. Alexis Carrel had verbally reported finding internal tumors in 18–30% of the members of an inbred line developed from animals of strain C 57 Black which he obtained in 1928. When this latter sub-strain, "Carrel's C 57 Black" was later examined cytologically its chiasma frequency, as recorded above, was found to be very low—26.7. A second test of chiasma frequency in Little's C 57 Black gave a result (29.7) not significantly different from that of the first test (30.3). Recently Dr. R. Korteweg of the Antoni van Leuvenhoek Laboratory, Amsterdam, reported verbally to the senior author that in his sub-strain of C 57 Black, which was obtained from Bar Harbor about four years ago, he finds about 10% of spontaneous leukemia, but no tumors. Dr. Little has since kindly supplied the information that no cases of leukemia have, however, been reported in C 57 Black at Bar Harbor.

It thus appears possible that there may be some genetic difference between C 57 Black and the other three resistant strains, A, D, and C-3-H. It is the strain used in the crosses in which Murray and Little (45, 46) have shown that susceptibility is transmitted predominantly by the female parent. The publication of detailed records of the behavior of other resistant strains in crosses with susceptible ones will be awaited with great interest.

One other strain, M Leaden, reported as having "an extraordinarily low cancer incidence" was found to have a chiasma frequency as low as any of the susceptible strains. After the first test had shown a frequency of 27.8 this result was in turn communicated to Dr. Little who in reply informed us that it is derived from the same stock as the other exception. It originated as a mutant in 1929–30 from the C 57 Brown strain, which in turn was segregated in 1924 from C 57 Black which Dr. Little started inbreeding in 1921. In the experience of Dr. Joseph Murray, who, according to Dr. Little has had most experience with it, the M Leaden strain has an "extraordinarily low" tumor incidence, as stated in the published leaflet, but a number of tumors have occurred in old females. A second test of the chiasma frequency of M Leaden gave a result (26.7) even lower than the first.

Our results may therefore be summarized as follows: (1) four strains (or five if Carrel's C 57 is included) reported to have a "high incidence" of spontaneous tumors and one strain having "tumors at an advanced age" all have low chiasma frequencies; (2) three strains having "no tumors observed" in ten or more generations have high chiasma frequencies; (3) two related strains which though classed as resistant have, either at Bar Harbor or elsewhere, a small proportion of tumors or leukemia at an advanced age and which belong to a stock which has produced a susceptible strain, have intermediate or low chiasma frequencies.

Until further detailed records of the various strains both in pure line and hybrid studies are available it is doubtless futile to make further attempts to discover whether or not there is a consistent relationship between chiasma frequency and tumor susceptibility. If no spontaneous tumors whatever

are found in strains N, I, and CBA, it appears that chiasma frequency may possibly give a more precise indication of genetic constitution in regard to cancer susceptibility—one less affected by environmental conditions and such factors as relative longevity—than direct tests. On the other hand, the discovery of tumors in these lines (or in sub-strains from them if unaccompanied by a characteristic change in chiasma frequency) would show that the present apparent general correlation lacks significance.

So far as present records go, there are, then, six susceptible strains with low chiasma frequencies, and three resistant with high frequencies, or a total of nine concordant cases, and there are two (related) discordant strains. Neither of the discordant strains is beyond suspicion with regard to its degree of resistance. This degree of concordance makes it highly improbable that the differences in chiasma frequency are caused by some unknown factor independent of tumor susceptibility or resistance, but does not, of course, entirely rule out that possibility.

If we assume for the present that there *is* a general correlation between low chiasma frequency and susceptibility to spontaneous occurrence of tumors, we may next consider its possible significance.* It seems to indicate, first, that in an organism susceptible to tumor formation the processes of chromosome "splitting", spindle formation and cytokinesis are not correlated normally or are near the borderline of lack of correlation. If so, then the condition of still more abnormal correlation found in tumor cells themselves may be due either to the loss of remaining regulatory genes or to their mutation. On any of the somewhat related, though in detail distinct, concepts of *(a)* orthogenetic gene variation, *(b)* premutation (cf. 24) or *(c)* genic "Grenzwerte" (47) the slight lack of correlation indicated by lower chiasma frequency would be due to the genes controlling and regulating mitosis being in part way mutated in susceptible strains. Or, control of mitosis may depend upon a number of genes and all but one of these be already mutated.

There are many cases of similar morphological changes being produced by either gross, readily visible, chromosome losses or deficiencies, and by "point" or gene mutations (neglecting as irrelevant for present purposes newer data on the nature of "point mutation"). Asynapsis also can be produced either by gene mutation or chromosome loss. It is therefore probable that if malignancy is due to somatic cell mutation it may be caused in some cases by gene mutation and in others by chromosome loss or deficiency. Mendelsohn (42), in a paper published since the above statement was first drafted, has shown that in both sectioned material and tissue cultures of cysticercus cysts of rat liver there are many abnormal mitoses. He concludes, "The onset of malignancy in the cysticercus cysts may be associated in some way with the presence of abnormal mitoses. Their presence seems to be a manifestation of a potential carcinogenic agent at work." Mustard and other non-carcinogenic irritants produced superficially somewhat similar inflammatory reactions, but no mitotic irregularities. This evidence, together with Mottram's (44) observation that certain carcinogenic agents cause chromo-

* *See footnote on page 58.*

some irregularities, emphasizes the probable significance of chromosome mutation. Considering first this possibility: irregularities of mitosis might be expected to occur with greater frequency in susceptible animals than in resistant ones having a greater margin of safety in the correlation of these processes. Through irregularities of mitosis, chromosomes or parts of chromosomes bearing genes essential for the regulation of mitosis could readily be lost (see also 31). Carcinogenic agents or any "irritant" or chronic injury might be the immediate cause of these irregularities. On the other hand, if malignancy arises through point mutation, then, on any of the concepts mentioned above, the controlling genes in susceptible strains would much more readily mutate further to the ineffective state than would the comparable genes, well within the "Grenzwert" of resistant strains. It is possible that induced tumors (including here the cysticercus cysts) may originate predominantly through gross chromosome changes whereas "spontaneous" tumors may more often be due to "gene mutation". This is a speculation for which there is at present no direct evidence, but critical comparative studies of the chromosomes in both types of tumor from the same susceptible strain might be made to test it. The whole problem of the relationship, if any, between susceptibility or resistance to "spontaneous" tumors and reactivity to artificially applied carcinogenic agents is only just now receiving attention. Andervont (1) found that strains A, C-3-H, and CBA are all susceptible to the carcinogenic action of 1 : 2 : 5 : 6- dibenzanthracene, but that strains A, C-3-H, and also D (all susceptible to spontaneous tumor formation) react much earlier than do CBA and his stock mice, both of which strains "develop very few spontaneous growths". Lynch (1935) showed that seven different strains of mice all showed high incidence of sarcoma at the site of injection following subcutaneous injection of 1 : 2 : 5 : 6- dibenzanthracene. On the other hand the incidence of lung tumors ranged from 0.0 to 89.1% in the different strains and those that had the higher incidences were the strains that also have relatively high spontaneous occurrence of lung tumors. Without doubt there is a relationship between susceptibility to spontaneous occurrence of tumors and reactivity to certain carcinogenic agents, but the nature of the relationship is not yet clear in detail.

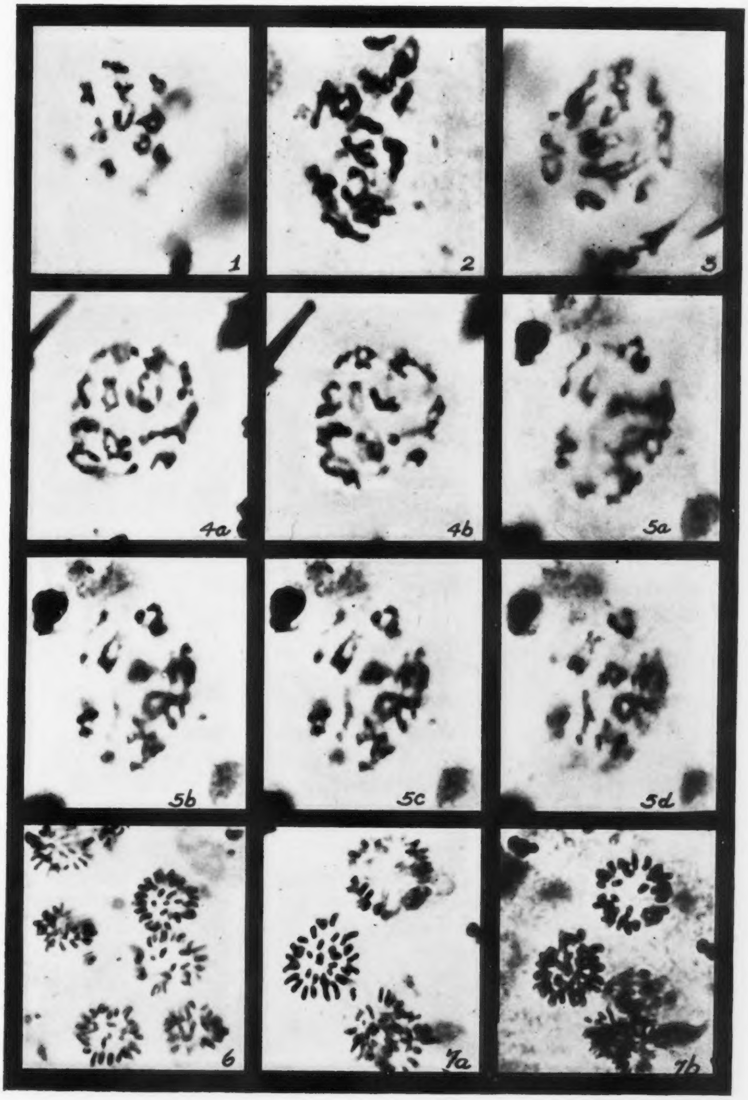
Lynch has shown also that susceptibility is organ-specific, and Potter and Richter (50) found that different lines of inoculated leukemias may, in susceptible mice, produce infiltrations in characteristically different situations.

If the correlation between chiasma frequency and tumor susceptibility is a general one, it almost certainly implies the existence of a generalized "cancer disposition". This concept is opposed by many leading workers in the field, who emphasize the specific nature and localization of tumors (or leukemias) in different strains. Without pressing the possible analogy, it may be pointed out that eversporting genes mutate with different frequencies in different tissues, and in the same tissues at different ages. The regular occurrence of malignancy in certain tissues may, however, be due to that tissue being the point of contact with the inciting stimulus, and hereditary susceptibilities

for localization and specificity may be as indirectly related physiologically to the primary susceptibility gene as are the modifying genes in Bonnevie's mice (9) which influence the distribution of blebs and thereby the ultimate effects of the X-ray induced mutant gene which causes syndactyly, abortive development of the feet, etc. The work of Curtis, Dunning and Bullock (16) shows that in their case it is the immediate inciting cause which is localized, and that the nature of the tumor depends upon its region of origin. From the present data it is suggested that what is inherited in tumor susceptible strains is a mutated state for *most* of the genes necessary for the control of mitosis, or a heightened tendency of controlling genes to be lost, to mutate, or to pass the "Grenzwerte". The medium of transmission of susceptibility on all but the first of these suggestions may be either the cytoplasm or the chromosomes, or both. Murray and Little (45, 46) have shown that in the cross of Dilute-brown dba (susceptible) × C57 Black, susceptibility is transmitted predominantly in the female line, and therefore, they consider extrachromosomally, but there is, they state, also evidence of chromosomal genes being involved. In a number of cases in plant hybrids chromosome pairing ("chiasma frequency") apparently depends on an interaction between the chromosomes and the cytoplasm—both may carry "factors" affecting it. Kostoff (32), by inducing polyploidy and consequent fertility in self-sterile *Nicotiana* hybrids, has shown that the tumors which occur in certain interspecific hybrids of this genus are heritable. Old plants have most tumors and in some cases the growing point may develop into tumors instead of branches. Kostoff notes that if tumors are due to somatic cell mutation, the mutation rate must be very high, and points out that some genes, for example that for red flower color, are known to be stable in the pure species in which they naturally occur, but highly unstable in hybrids. In species hybrids the paternal genes are of course in "foreign" cytoplasm. The danger of concluding that maternal transmission is necessarily plasmatic inheritance has, however, been strikingly shown by Dobzhansky (19).

The differences in chiasma frequency may, however, be only a symptom of differences in cellular metabolism, in which case the chromosome behavior is a primary concomitant of some underlying cause of tumor susceptibility. Since differences in cellular metabolism might possibly be revealed by differences in nucleolar size, studies of this were made, but we were unable to determine any difference in this respect between susceptible and resistant strains. Direct tests could, of course, be made where the necessary apparatus is available.

Resistance to the growth of implanted tumors or leukemias appears at present to be a completely distinct problem from the origin of neoplasms. That induced protection may be determined by the genetic constitution of the immunizing tissue is strikingly demonstrated by MacDowell, Taylor, and Potter (41) in an experiment of remarkable precision. As far as can be seen at present, however, this phase of the cancer problem is unrelated to the aspects of it that we are primarily concerned with in the present work.



Photomicrographs: FIGS. 1–5. *Diakinesis in primary spermatocytes, ca. 2000×.* FIGS. 6 AND 7. *Mitotic metaphases in spermatogonial cells, ca. 1500×.* FIG. 1. *C57 Black.* FIG. 2. *Carrel's derivative of C57 Black.* FIG. 3. *Strain I.* FIGS. 4A AND B. *M Leaden, successive focal levels.* FIGS. 5A, B, C AND D. *Strain A, successive focal levels.* FIG. 6. *C57 Black.* FIGS. 7A AND B. *Y, black, successive focal levels.*

Studies of somatic chromosomes in strains of mice susceptible and resistant to spontaneous tumors are being continued by the authors, at present independently, and by others in the hope that, with different techniques and tissues or different standards, it may be possible to assess on a more satisfactory basis the differences, if such are proved to be real, which at present can be referred only to a general impression. If these efforts are successful it may be possible to diagnose susceptibility by biopsy in advance of the occurrence of tumors. For the present, this study, being on spermatocytes, can have only indirect significance for the cancer problem in man. It is our hope that it may contribute to an understanding of the mechanism connecting hereditary transmission of tumor susceptibility and the origin of tumors.

Acknowledgments

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Appendix

A copy of the foregoing paper was submitted to Dr. C. C. Little, before publication, with a request for criticism and any new data available on the cancer history of the mice used. The data and information which Dr. Little supplied (January 21, 1936) are here appended rather than incorporated in the text for reasons which will become obvious in the presentation of them.

Strain C 57 Black now has two recorded instances of carcinoma of the breast. To the description of Strain Y may now be added that it has a low to medium incidence of breast tumors. One tumor has now been observed in Strain N. Strain C 57 Brown is now classed as having medium tumor incidence. All these are obviously minor additions to the records which do not change the general classification of the strains. A more serious alteration has occurred in the behavior of Strain CBA. From having for many years had no tumors of any description, it has "as the number of animals and number of generations of inbreeding increased" changed to the point where it must be considered as having a "low to medium tumor incidence." Dr. Little states that he and his co-workers have come to doubt whether there is any such thing as a cancer-free strain. As he says, "in view of our knowledge of the process of somatic mutation . . . . . . it would be surprising if sporadic mutation which might possibly give rise to a tumor did not occur." At the same time one

wonders if the change in a strain such as CBA may not be due in part at least to segregation or mutation having changed its general constitution. In any case this change does not materially affect our general conclusions since CBA must still be classed as resistant. Further, all mice of Strain CBA cytologically examined were obtained from Dr. Little between October 1933 and April 1934, when it was still classed as tumor-free, which may be significant with regard to the question of whether a change has occurred in the strain. The chiasma frequency was 0.88 lower in the second test than in the first of this strain. The animal used in the second test was obtained six months later than the first. But even if this difference were considered as possibly significant, the fact that the count was made on a single animal and that one four months older than any in the first test, would prevent any conclusion being reached from it. It may, however, be worth while to make counts on young animals of this strain now.

Most significant for the present study are Dr. Little's comments on Strain C 57 Black. Of it he states: "We are getting fairly clear evidence that although C 57 Black does not itself give a high incidence of tumors, hybrids derived from it and other strains have in some cases given higher tumor incidence than would be expected if the strain C 57 Black was entirely devoid of genetic tendency to tumor formation." He notes also that Korteweg (1934, Nederl. Tigds. Geneeskunde 78 : 240-245) has shown it to react more positively to certain carcinogenic agents than the dilute brown strain does. There are thus further indications that our apparent exception in the correlation of low chiasma frequency with susceptibility may in the course of further work prove to be highly significant.

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**Even were the data entirely in agreement with expectation based on the initial working hypothesis (outlined briefly in the Abstract) that would not, of course, establish its validity. That it has enabled us to discover interesting differences in chiasma frequency is for the present sufficient merit whatever the ultimate decision regarding its validity as an explanation of the hereditary mechanism involved in cancer susceptibility may be. Says Bertrand Russell:*

"The fact is that it is difficult to think of the right hypothesis, and no technique exists to facilitate this most essential step in scientific progress. Consequently, any methodical plan by which new hypotheses are suggested is apt to be useful; and if it is firmly believed in, it gives the investigator patience to go on testing continually fresh possibilities, however many may have previously had to be discarded".